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13. ABSTRACT (Maximum 200 Words)
The purpose of the proposal is to characterize global hemodynamics and microcirculatory oxygen transport in skeletal muscle in an animal model of severe (40 mm Hg), prolonged (4 hours) hemorrhagic hypotesion (HH). The target pressure and length of hemorrhage were set in the Army's original request for proposals. Arterial and venous blood pressures, gases, acid-based status, glucose, lactate, electrolytes, hemoglobin, O_2 delivery and consumption were measured before and up to 4 h after HH (mean arterial pressure, MAP = 40 mm Hg.) Ringer's lactate (RL) was used to maintain MAP. Fifty-three percent of rats survived $\geq$3 h (survivors, S); others were considered non-survivors (NS). Following HH, all rats required RL infusion for $\geq$ h. NS showed a significantly greater degree of metabolic acidosis that S. Respiratory rate, arterial PO_2, O_2Sat, O_2 content, glucose and pH were significantly higher in S. Rate of RL infusion, arterial K+ and PCO_2 were lower in S. Arterial K+ and RR were the only parameters significantly different between S and NS at all time points during HH. The data suggest that early oxygenation and metabolic compensation are essential for survival of prolonged HH.

14. SUBJECT TERMS
Hemorrhagic shock, microcirculation, skeletal muscle, hemodynamics, oxygen, rodent

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INTRODUCTION

The subject of this research project involves cardiovascular responses to severe, prolonged hemorrhage. The purpose of the funded proposal was to characterize both global hemodynamics and skeletal muscle microcirculatory oxygen transport in an animal model of severe (mean arterial pressure = 40 mm Hg), prolonged (four hours) hemorrhagic shock. The primary model parameters (target pressure and length of hemorrhage) were set in the Army's original request for proposals. Based on proposal feedback and discussions with the Program Officer, we received approval to switch from the hamster to the rat as the experimental animal used in this project. The simultaneous measurement of macrocirculatory (i.e., systemic) and microcirculatory variables is unique in this study and this has been one of the challenging, as well as rewarding, aspects of the project.

BODY

We experienced initial delays in hiring personnel with the appropriate technical and surgical backgrounds for performing the difficult microcirculatory studies and the implantation of the aortic flow probe. We were fortunate to obtain the services of Dr. Ivo Torres Filho and Ms. Luciana Torres, both of whom have extensive experience in this research area. After consultation with the Program Officer for this project, we made the decision to change the animal species used in this study from hamster to rat. Initial experiments were designed to optimize the implantation of the aortic flow probe in order to avoid poor cardiovascular responses to hemorrhage due to instrumentation. The aortic flow probe was implanted, under sterile conditions, in each animal 5-8 days before experimentation. All techniques for blood sampling and analysis, central hemodynamic quantification and microvascular preparation were worked out in detail, also, during this initial period. Simultaneous measurements of systemic and microcirculatory parameters were carried out, as well as analysis of blood gases and chemistry. We have used the BioPac system for continuous data acquisition of macrohemodynamic variables: cardiac output, CO; heart rate, HR; arterial pressure, AP (including systolic pressure, SP; diastolic pressure, DP; pulse pressure, PP); central venous pressure, CVP; respiratory rate, RR. Blood samples were analyzed immediately after withdrawal using a Radiometer ABL 705 and a Radiometer OSM3 in the same room where the experiments were conducted. The OSM3 Hemoximeter was set up specifically for rat blood to yield the following quantities: total Hb, deoxy-Hb, HbO_2, O_2 saturation, HbCO, metHb and O_2 content.

The animals were heavily instrumented for an experiment, due to the large number of variables measured, and a listing of the various cannulations, their uses and the quantities obtained are:

aortic flow probe - CO, HR, SV (stroke volume)
carotid and femoral arteries - AP, DP, SP, PP, hemorrhage, ABG (arterial blood gas)
jugular and femoral veins - CVP, RR, continuous anesthetic infusion, VBG (venous blood gas)
tracheostomy
temperature probe for core temperature monitoring (esophageal)
spinotrapezius preparation - microvessel diameter, hemoglobin oxygen saturation

The rat spinotrapezius muscle was used as the preparation for intravital video microscopic studies. The microcirculatory data were collected as video images that were recorded on videotape. These images were then analyzed off-line to yield values for microvascular diameter, oxygen saturation and hemoglobin concentration. This has been a very labor-intensive part of the analysis, and we have developed procedures to streamline the process. The amount of time required for an experiment was approximately 12 hours (preparation, data collection, cleanup).

The data from the systemic measurements are included in Tables 1 – 6 and Figures 1 – 9 in the full manuscript included in the Appendix. The following is a listing of the measured and calculated parameters in this study as they appear in Figures 1 – 9 of the enclosed manuscript:

Figure 1. Left panel. Total *volume of hemorrhaged blood* followed by cumulative *volume of Ringer's lactate solution infused* to maintain MAP at 40 mm Hg. **Right panel.** *Venous hemoglobin concentration* at various time points before and during hemorrhagic hypotension.

Figure 2. *Total peripheral resistance*

Figure 3. Panel A. *Respiratory rate;* **Panels B-D.** *Arterial Hb O_2 saturation, PO_2 and O_2 content*

Figure 4. *Arterial blood levels of base excess, HCO_3^- and lactate*

Figure 5. *Arterial blood levels of pH and PCO_2*

Figure 6. *Systemic VO_2* (oxygen consumption) as a function of *systemic DO_2* (convective oxygen delivery)

Figure 7. Panel A. *Difference between mean calculated VO_2 and mean baseline VO_2.* **Panels B and C.** *O_2 debt and the cumulative O_2 debt*

Figure 8. *Original tracings* of a typical experiment illustrating the rapid death experienced by animals during prolonged hemorrhagic hypotension. From top to bottom: *mean arterial pressure, central venous pressure, aortic blood flow, mean arterial blood pressure and mean aortic flow.*

Figure 9. *Arterial (panel A) and venous (panel B) plasma potassium levels. Arterial (panel C) and venous (panel D) glucose levels.*

After an animal was prepared for both microcirculatory and macrocirculatory measurements, two sets of baseline values were collected and then the hemorrhage protocol was carried out, so that blood was withdrawn from the animal until mean arterial pressure reached 40 mmHg. The time after hemorrhage was referenced to this point (i.e., "time zero" occurred when 40 mmHg was reached) and the arterial pressure was monitored; additional blood was withdrawn if pressure rose above 45 mmHg, and fluid (Ringer's Lactate, RL) was infused if pressure fell below 35 mmHg, in order to maintain the arterial pressure at or near 40 mmHg for the duration of the hypotensive state.

In regard to fluid infusion, all animals required RL infusion to maintain arterial pressure at 40 mmHg for one hour or longer (Fig. 1, manuscript). No animal required RL infusion of more than 2.5 times the hemorrhaged volume. The hemorrhaged volume for survivors was 28.3 ± 3.2 ml/kg; for non-survivors it was 30.3 ± 3.2 ml/kg.

Our data indicate that the mortality in this model is 47% using 3 hours as the survival time. In the results that follow, we will take survival for 3 or more hours as our definition of a "survivor."

We observed that HR decreased continuously throughout the hypotensive period at the same rate in all animals (i.e., survivors and non-survivors; Table 5). Similar behavior for both survivors and non-survivors were observed in CO, CI (Table 5), HR (Table 5), CVP (Table 4), BE (Fig. 4), pH (Fig. 5), and arterial PCO_2 (Fig. 5). The peak increase in TPR (for both survivors and non-survivors) was observed within the first 30 min into the hypotensive period (Fig. 2). The peak increase in lactate (for both survivors and non-survivors) was observed at 120 min into the hypotensive period (Fig. 4). All figures and tables refer to those in the manuscript included in the Appendix.

Significant differences ($P < 0.05$) between survivors and non-survivors were obtained for the following variables:

- respiratory rate (higher in survivors).
- arterial PO_2, O_2 saturation, glucose and pH (higher in survivors).
- arterial K^+ and PCO_2 (lower in survivors)
- small arteriolar diameter (constriction in survivors)
- large arteriolar diameter (dilation in survivors)

- infusion of RL (slower in survivors)

Systemic Studies

Male Sprague-Dawley rats (250-300 g) were anesthetized with Isoflurane, intubated and mechanically ventilated with a rodent pressure-controlled ventilator. Following sterile thoracotomy, a transit-time flowmeter probe was placed around the aorta for continuous measurement of cardiac output. The chest was closed and animals were allowed a 5-8 day recovery period. The implanted animals were submitted to a tracheostomy and catheterizations under ketamine/acepromazine followed by constant i.v. infusion of Alfaxalone/Alfadolone acetate (Saffan, Schering-Plough Animal Health). The right carotid artery and right jugular vein were used to measure continuously MAP and CVP, respectively; in addition, the right femoral artery and left femoral vein were used for blood sampling and Saffan infusion, respectively. All catheters were flushed occasionally with heparinized saline to inhibit clotting in the catheter, but this procedure delivered insignificant amounts of heparin to the animal. The core temperature was monitored and maintained at 37 °C by a heating blanket in the animal platform.

Physiological parameters included MAP, CVP, heart and respiratory rates, total peripheral resistance, stroke index, cardiac output, DO_2 and VO_2. In addition, arterial and venous PO_2, PCO_2, lactate, pH, hemoglobin concentration and O_2 saturation (O_2Sat) were determined. Global VO_2 was calculated by using Fick's principle as the product of cardiac index (CI) and the difference between arterial (CaO_2) and venous (CvO_2) O_2 contents: $CaO_2 = (Hb * 1.39 * SaO_2) + (0.003 * PaO_2)$, $CvO_2 = (Hb * 1.39 * SvO_2) + (0.003 * PvO_2)$, $VO_2 = CI * (CaO_2 - CvO_2)$, in which SaO_2 and SvO_2 are the arterial and venous O_2 saturations, respectively, and PaO_2 and PvO_2 are the arterial and venous O_2 tensions, respectively. Whole animal DO_2 was computed as the product of CaO_2 and CI.

After the systemic control conditions of the animals were assessed, shock was induced by arterial blood withdrawal until MAP reached 40 mmHg. Measurements were made before and up to 4 h into the hemorrhagic hypotension (HH) period. Additional hemorrhage or Ringer's lactate (RL) infusion was used to maintain MAP at 40 mmHg. Control rats were subjected to the same procedures except HH. Except for a small hemodilution (due to blood sampling for analysis and fluid replacement with RL), these control animals did not show significant changes in systemic parameters over time (see Tables 1-3; manuscript in Appendix). In hemorrhaged animals, 53% of rats survived 3 hours or longer (survivors, S); rats surviving less than 3 hours were considered non-survivors (NS). All rats required RL infusion for at least one hour (up to 3 times the hemorrhage volume).

Microvascular Studies

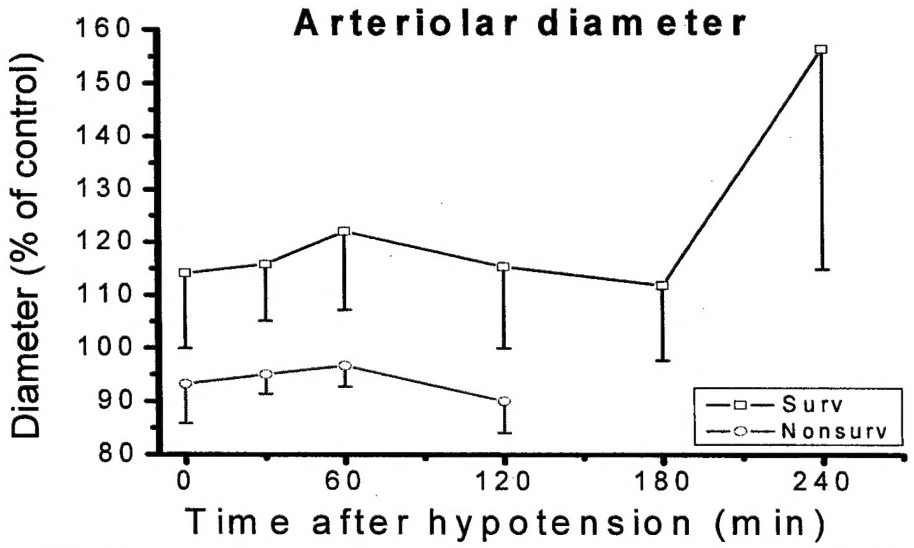

Figure 1. Diameter (in % of baseline) of large arterioles obtained from 4 survivors and 4 non-survivors.

We combined measurements of central hemodynamics and systemic oxygenation with microvascular observations. This allowed us to determine if any macrocirculatory or systemic alterations correlated with microcirculatory alterations in survivors and non-survivors.

Male Sprague Dawley rats were first instrumented as described above. The experiments were performed on the spinotrapezius muscle following a 5-8 day recovery period after the flow probe implantation. The spinotrapezius muscle was prepared for intravital microscopic viewing with transmitted or incident light. The spinotrapezius muscle was dissected from its medial attachments and placed over a warmed Plexiglas viewing platform. After dissection, the muscle was covered with a thin plastic film (Saran Wrap) to minimize both desiccation of the tissue and gas exchange with the atmosphere. We did not observe any obvious deleterious effects of Saran (e.g., altered microvascular patency, platelet aggregation, or increased numbers of white blood cells) on the exteriorized spinotrapezius muscle. We measured functional capillary density, as well as internal diameters.

The same protocol described above in the "Systemic Studies" was used in these combined microvascular experiments. After a control period, mean arterial pressure was decreased to 40 mmHg by controlled hemorrhage. Measurements were made before and up to 4 h into the HH period. Control rats were subjected to the same procedures except HH. These control animals did not show significant changes over time, supporting the concept that systemic and microvascular measurements can be acquired in the same

experiment. At the microvascular level, venules and small arterioles (less than 35 μm in diameter) constricted, whereas large arterioles dilated in S (Figure 1 above). This finding, along with the systemic data, supports the concept that differences between survivors and nonsurvivors can be found at the level of the microcirculation.

Problems: We underestimated the amount of time and effort required to process and analyze the large number of images collected to provide data on microcirculatory variables: diameter, functional capillary density (FCD), hemoglobin concentration ([Hb]) and oxygen saturation (SO_2). We discovered that the quality of the microcirculatory images became degraded with the progression of hemorrhagic hypotension (HH), going from quite sharp and crisp during the control and early HH to poor in the later stages of the HH period. This degradation in image quality appears to be due to the increased interstitial edema brought on by the need to infuse large volumes of RL to maintain MAP near 40 mmHg. The importance of images with high optical clarity is especially critical for the determination of FCD (due to the small size of the capillaries) and the photometrically determined oxygenation variables [Hb] and SO_2. All of the images have been collected, and we have analyzed most of them; however, we have found that much greater care (with corresponding increase in time and effort) is needed to obtain reliable results from these images, due to their poorer quality. Although this problem has delayed the completion of this aspect of the project, the image analysis will continue until completed. The data from this part of the project have not been included in this report, due to their incomplete state, but they will be forwarded as soon as they are available, by approximately the end of December 2003.

Recommendations: As seen in Fig. 1 of the enclosed manuscript, a large volume of fluid must be infused into the hemorrhaged animals in order to maintain arterial pressure at 40 mmHg. We briefly explored another option whereby a single (or double) volume hemorrhage brought arterial pressure down to 50 mmHg and below for up to 4 hours without any re-infusion of fluid. Maintaining the MAP slightly above 40 mmHg (45-50 mmHg) in this way may require less RL infusion to keep animals alive for several hours. We discussed this possible change in hemorrhage protocol with the Program Officer, but together we decided not to implement this modification in the current study.

Bibliography:

<u>Abstracts/Presentations:</u>

Torres, L.N., Torres Filho, I.P., Tiba, M., Barbee, W., Ward, K., Ivatury, R., and Pittman, R. *Small animal model for studying microcirculatory and systemic responses to prolonged hemorrhagic hypotension.* ATACCC, St. Pete Beach, Florida, 2002.

Torres, L.N., Torres Filho, I.P., Tiba, M., Barbee, W., Ward, K., Ivatury, R., and Pittman, R. *Microcirculatory and systemic responses to hemorrhagic shock.* ATACCC, St. Pete Beach, Florida, 2002.

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Full manuscript:

Torres, L.N., Torres Filho, I.P., Barbee, W., Tiba, M., Ward, K., and Pittman, R. *Systemic responses to prolonged hemorrhagic hypotension (HH).* Submitted to American Journal of Physiology, 2003.

Personnel receiving pay from research effort:

R.W. Barbee
A.S. Golub
R.R. Ivatury
R.N. Pittman (P.I.)
I.P. Torres Filho
L.N. Torres
K.R. Ward

KEY RESEARCH ACCOMPLISHMENTS

- Simultaneous measurements of macro- and microcirculatory variables

Differences between survivors and non-survivors were obtained for:

- Respiratory rate (higher in survivors)
- Arterial PO_2, O_2 saturation, O_2 content, glucose and pH (higher in survivors)
- Small arteriole diameter (constriction in survivors)
- Large arteriole diameter (dilation in survivors)
- Rate of infusion of RL, arterial K^+ and PCO_2 (slower in survivors)

REPORTABLE OUTCOMES

Scientific Presentations:

Torres, L.N., Torres Filho, I.P., Tiba, M., Barbee, W., Ward, K., Ivatury, R., and Pittman, R. *Small animal model for studying microcirculatory and systemic responses to prolonged hemorrhagic hypotension.* ATACCC, St. Pete Beach, Florida, 2002.

Torres, L.N., Torres Filho, I.P., Tiba, M., Barbee, W., Ward, K., Ivatury, R., and Pittman, R. *Microcirculatory and systemic responses to hemorrhagic shock.* ATACCC, St. Pete Beach, Florida, 2002.

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Scientific Publications – Abstract:

Torres, L.N., Torres Filho, I.P., Tiba, M., Barbee, W., Ward, K., Ivatury, R., and Pittman, R. *Systemic and microvascular responses to prolonged hemorrhagic hypotension (HH).* Experimental Biology Meeting, San Diego, California, 2003.

Scientific Publications – Full manuscript:

Torres, L.N., Torres Filho, I.P., Barbee, W., Tiba, M., Ward, K., and Pittman, R. *Systemic responses to prolonged hemorrhagic hypotension (HH).* Submitted to American Journal of Physiology, 2003.

Grant application based on this work:

Microvascular Responses to Hemorrhage and Resuscitation, National Institutes of Health, submitted May 23, 2003, R.N. Pittman, P.I. (awaiting funding decision)

CONCLUSIONS

We have carried out experiments in an animal model of prolonged hemorrhagic hypotension (HH) to an arterial pressure of 40 mmHg, performing simultaneous macro- and microcirculatory measurements of hemodynamic, oxygenation and blood chemistry variables. We have identified trends in several variables that distinguish survivors (> 3 hours) from non-survivors (< 3 hours). Non-survivors showed a significantly greater degree of metabolic acidosis than survivors. Respiratory rate, arterial PO_2, O_2Sat, O_2 content, glucose and pH were significantly higher in survivors. Rate of RL infusion, arterial K^+ and PCO_2 were lower in survivors. Arterial K^+ and RR were the only parameters significantly different between survivors and non-survivors at all time points during HH. Arterial levels of K^+ showed the clearest distinction between S and NS and may explain the sudden death experienced by animals during HH. The data suggest that early oxygenation and metabolic compensation are essential for survival of prolonged HH. We have found that this particular model of hemorrhage requires a large amount of

fluid to be re-infused into the animal. Because of this finding, we recommend that consideration be given to other hemorrhage protocols that would be more in line with circumstances that might occur under combat conditions where limited volumes of fluid would be available for infusion. The results of this study should be able to provide valuable guidance in regard to triage of combat casualties.

REFERENCES

None

APPENDICES

Presentations:

Torres, L.N., Torres Filho, I.P., Tiba, M., Barbee, W., Ward, K., Ivatury, R., and Pittman, R. *Small animal model for studying microcirculatory and systemic responses to prolonged hemorrhagic hypotension.* ATACCC, St. Pete Beach, Florida, 2002.

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Abstract:

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Full Manuscript (in review):

Torres, L.N., Torres Filho, I.P., Barbee, W., Tiba, M., Ward, K., and Pittman, R. *Systemic responses to prolonged hemorrhagic hypotension (HH).* Submitted to American Journal of Physiology, 2003.

Small Animal Model for Studying Microcirculatory and Systemic Responses to Prolonged Hemorrhagic Shock

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INTRODUCTION

The combat mortality rate has averaged about 20% over the last 60 years. Approximately one-fifth of these deaths are potentially preventable and primarily due to hemorrhage.

Hemorrhagic shock is due to inadequate tissue perfusion and removal of cellular waste products which cause defects of oxygen delivery, transport or utilization.

Since the hemorrhagic shock in the military setting is often complicated by delays in transport of injured personnel to medical facilities, intermittent monitoring of the microcirculation could be valuable in assessing which soldiers are most critically injured.

AIM

Develop a stable and relevant model to characterize microcirculatory and systemic responses in rats during severe and prolonged hemorrhagic shock, avoiding excessive mortality and deterioration in central or microvascular hemodynamics during the five hours of experimental period.

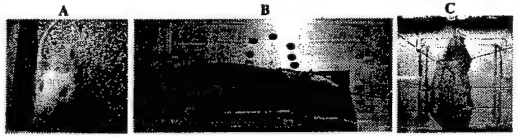
METHODS

Under sterile conditions, male Sprague-Dawley rats were implanted with transit-time transducers on the ascending aorta (for monitoring the cardiac output, CO) using isoflurane anesthesia and a pressure controlled ventilator. After 5-8 days, rats recovered to at least 90% of body weight on day of implantation and were anesthetized with ketamine/acepromazine, until vein access was obtained for Saffan infusion. The femoral artery was cannulated for continuous measurement of mean arterial blood pressure (MAP).The carotid artery was cannulated for blood removal and the jugular vein was cannulated for central venous pressure (CVP). The core temperature of the animal was maintained at 37ºC. The spinotrapezius muscle was prepared for intravital microscopy, placed on a heated platform and covered with Saran to minimize both tissue desiccation and exposure to atmosphere. Arteriolar and venular diameters were determined using video recorded images. Blood gases and chemistry were measured using a Radiometer ABL705 multi-profile and a Hemoximeter set up specifically for rat blood. All systemic parameters were continuously stored in computer via data acquisition system. Rats were heparinized with 4units/ml. Normovolemic animals were used as controls.

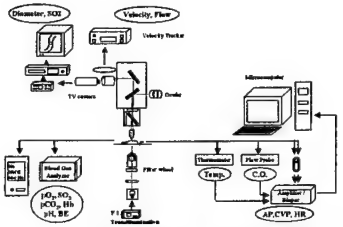
PROLONGED HEMORRHAGIC HYPOTENSION

Tested protocols

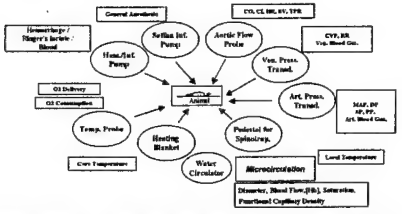
- ✓ **Using blood as infusion fluid:**
  - * 1h at 40 mmHg + 3h at 60 mmHg [H40/60]
  - * 4h at 40 mmHg [H 40 B]
- ✓ **Using Ringer's lactate as infusion fluid:**
  - * 4h at 40 mmHg [H 40 RL]
- ✓ **Fixed volume hemorrhage:**
  - * 4h at ≤ 50 mmHg



Panel A: Unanesthetized rat 7 days after aortic flow probe implantation. Panel B: Exteriorized rat spinotrapezius muscle preparation. Instrumented animal over a thermo-controlled blanket with the muscle placed on the heated platform. Panel C: close-up of the muscle covered with a plastic film.

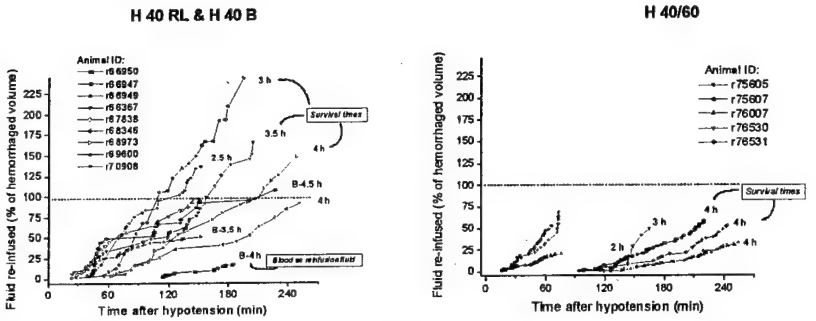


Schematic drawing of the system used to simultaneously collect microcirculatory and systemic data.

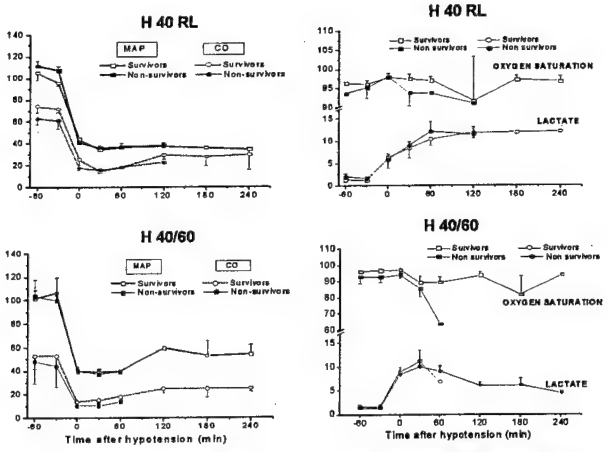


Schematic drawing of all parameters. Monitored: MAP, CO, CVP, heart rate (HR), hemorrhaged and re-infused volumes, arterial and venous pO2, pCO2, saturation, Hb concentration ([Hb]), lactate, base excess, pH, arteriolar and venular diameter and saturation. Calculated: stroke volume (SV), total peripheral resistance (TPR), diastolic (DP), systolic (SP) and pulse (PP) pressures, cardiac index (CI), respiratory rate (RR), O2 delivery and consumption.

RESULTS

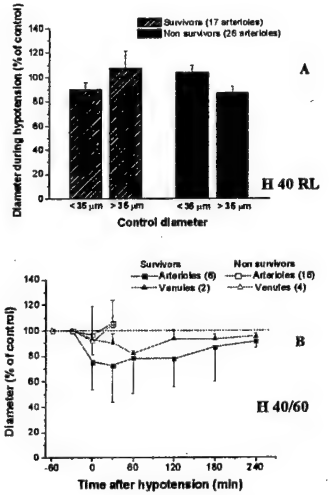


Volume of re-infused fluid in percent of the total shed blood. Panel A, rats received RL or blood infusion to keep the AP at 40 mmHg for 1 h or longer. Panel B, rats received blood to keep the AP at 40 mmHg for 1 hour and 60 mmHg for 3 hours.



Mean arterial pressure and cardiac output as a function of time obtained from 2 different protocols.

Arterial Oxygen saturation and lactate as a function of time obtained from 2 different protocols.



Microvascular diameter during hypotension.
Panel A, small and large arterioles from 4 survivors and 4 non survivors.
Panel B, arterioles and venules from 4 survivors and 3 non survivors.

CONCLUSION

The developed model allows identifying microcirculatory and systemic temporal alterations in all these different protocols, and helps to determine differences between survivors and non-survivors during severe and prolonged shock.

Effects of Prolonged Hemorrhagic Hypotension (HH) on Systemic and Microcirculatory Parameters

L.N. Torres, I.P. Torres Filho, M.H. Tiba, R.W. Barbee, K. Ward, R. Ivatury and R.N. Pittman

Virginia Commonwealth University • School of Medicine, Departments of Physiology, Anesthesiology, Emergency Medicine • VCU Reanimation Engineering Shock Center, Richmond, VA

Introduction

The combat mortality rate has averaged about 20% over the last 60 years, and 1/5 of these deaths are primarily due to hemorrhage.

Hemorrhagic shock is due to inadequate tissue perfusion and removal of cellular waste products, which cause defects of oxygen delivery, transport or utilization.

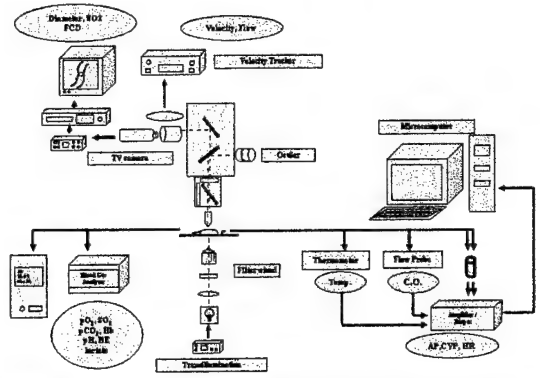
Since hemorrhagic shock is often complicated by delays in transport of injured soldiers to medical facilities, intermittent monitoring of the microcirculation, hemodynamics and oxygenation parameters could be valuable in assessing which soldiers are most critically wounded.

Aim

Determine microvascular and systemic differences between rats survivors and nonsurvivors during 4-hour HH (hemorrhagic hypotension) in rats, mimicking mortality and morbidity in the battlefield.

Methods

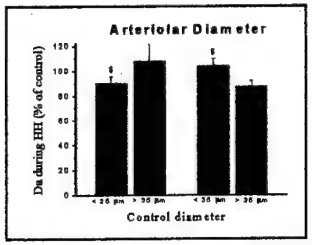
Under sterile conditions, 21 male Sprague-Dawley rats were implanted with transit-time transducers (Transonic Systems) on the ascending aorta (for monitoring the cardiac output, CO) using isoflurane anesthesia (1-3%) and a pressure controlled ventilator. After 5-8 days, rats recovered to the body weight of day of implantation and were anesthetized with isoflurane (2%) until vein access was obtained for Saffan infusion. The femoral artery was cannulated for continuous measurement of mean arterial blood pressure (MAP). The carotid artery was cannulated for blood removal and the jugular vein was cannulated for central venous pressure (CVP) measurement. The core temperature of the animal was maintained at 37°C. The spinotrapezius muscle was prepared for intravital microscopy, placed on a heated platform and covered with Saran to reduce tissue desiccation and exposure to atmosphere. Microvascular diameter and functional capillary density (FCD) were determined from video recorded images. Blood gases and biochemistry were measured using a Radiometer ABL705 and a Hemoximeter set up specifically for rat blood. Rats were heparinized (240 units/kg). All systemic variables were continuously stored in computer via data acquisition system. Normovolemic rats were used as controls. Fifty different parameters were compared between S and NS.



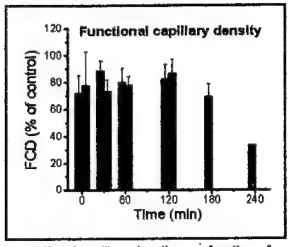
Schematic drawing of the system used to simultaneously collect microcirculatory and systemic data.

Results

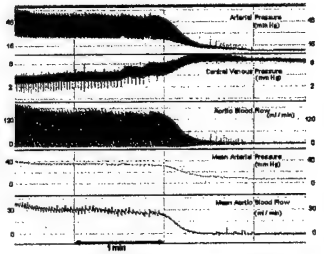
Data are expressed in mean ± SEM. S and NS represent survivors and non-survivors, respectively. # = significantly different from the control group at same time point * = significantly different from S ; $ = significantly different from large arterioles for both S and NS. A level of $p<0.05$ was consider significant.



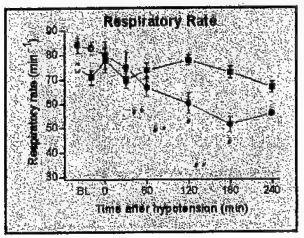
Diameter of small and large arterioles obtained from 4 S (blue) and 4 NS (red).

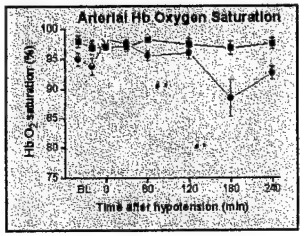


Functional capillary density as a function of time obtained from 4 S (blue) and 5 NS (red).

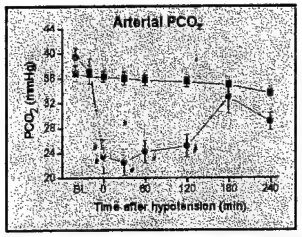


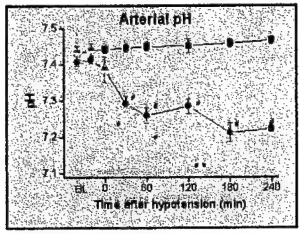
Original tracings of typical experiment illustrating the rapid death experienced by animals during prolonged HH. From top to bottom: mean arterial pressure, central venous pressure, aortic flow, mean arterial pressure and mean aortic flow.



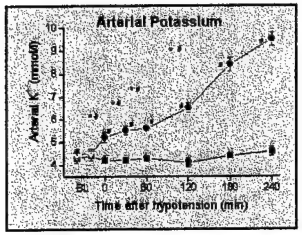


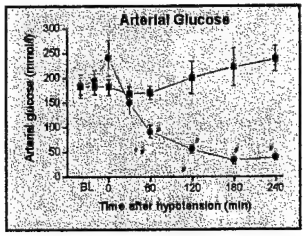
RR and Hb O2 sat as a function of time obtained from 9 S (blue), 8 NS (yellow) and 4 controls (black).





PCO_2 and pH as a function of time obtained from 9 S (blue), 8 NS (yellow) and 4 controls (black).





K^+ and glucose as a function of time obtained from 9 S (blue), 8 NS (yellow) and 4 controls (black).

Conclusions

1) S showed better oxygenation responses to HH than NS by having higher pO_2, Hb O_2 Sat and O_2 content; 2) better ventilation may explain these findings since S showed higher RR than NS throughout HH; 3) S and NS animals died during HH under high plasma K^+ and lactate, low glucose, Ca^{++}, BE and pH; 4) S showed a tendency to lower O_2 debt than NS; 5) the rate of infused RL (to keep MAP at 40 mm Hg) was higher in NS; 6) small arterioles constricted in S. Arterial plasma K^+ and RR were the only parameters significantly different between S and NS at all time points, suggesting that early oxygenation and metabolic compensation are essential for the survival to HH. The acute cardiorespiratory failure leading to death seems to be mainly due to severe hyperkalemia.

Systemic and microvascular responses to prolonged hemorrhagic hypotension (HH)

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Since microvascular studies provide limited information on systemic data, we developed a model to assess the effects of HH on microvascular, biochemical and systemic parameters. An aortic transducer was implanted for continuous measurement of cardiac output. After 5-8 days experiments were performed on the spinotrapezius muscle. Physiologic parameters were arterial pressure (AP), central venous pressure (CVP), heart and respiratory rates, arterial and venous pO_2, pCO_2, lactate, base excess, pH, [Hb], O_2 saturation (O_2Sat), total peripheral resistance (TPR), O_2 delivery (DO_2), functional capillary density, diameter and O_2Sat of arterioles and venules. Measurements were made before and up to 4 h after HH (40 mmHg). Additional bleeding or lactated Ringer's (LR) infusion were used to maintain HH. Controls were subjected to the same procedures except HH. About 67% of rats survived ≥3 h (survivors, S); others were considered non-survivors (NS). All rats required LR infusion for ≥1 h (up to 3 times the bleed volume). Significant differences between S and NS were obtained for DO_2, CVP, lactate, [Hb], p_vCO_2, p_aO_2, O_2Sat and pH (all higher in S). Rate of LR infusion, TPR, p_aCO_2, systolic and pulse pressures were significantly lower in S. Venules and small arterioles constricted, while large arterioles dilated in S. In conclusion, comparisons of S vs. NS may enable us to select variables for clinical monitoring and to evaluate treatment schemes for HH.

Supp: DOD and CNPq

Systemic Responses to Prolonged Hemorrhagic Hypotension

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Running head: Systemic responses to prolonged hemorrhagic hypotension.

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The material presented in this report is original and has not been submitted for publication elsewhere other than in abstract form.

ABSTRACT

Studies are needed to provide a rigorous examination of the relevance of monitored variables during prolonged hemorrhagic hypotension (HH). This study was designed to investigate the parameters that describe biochemical and O_2 transport patterns in animals subjected to HH. Systemic parameters that could differentiate survivors from nonsurvivors were identified. An aortic flow probe was implanted in rats (n = 21) for continuous measurement of cardiac output. Experiments were performed 6-9 days after surgery. Arterial and venous blood pressures, gases, acid-base status, glucose, lactate, electrolytes, hemoglobin, O_2 saturation (O_2Sat), heart and respiratory (RR) rates, total peripheral resistance, O_2 delivery and consumption were measured before and up to 4 h after HH (mean arterial pressure, MAP = 40 mm Hg). Ringer's lactate (RL) was used to maintain MAP. Fifty-three percent of rats survived ≥3 h (survivors, S); others were considered non-survivors (NS). Following HH, all rats required RL infusion for ≥1 h. Nonsurvivors showed a significantly greater degree of metabolic acidosis than survivors. Respiratory rate, arterial PO_2, O_2Sat, O_2 content, glucose and pH were significantly higher in S. Rate of RL infusion, arterial K^+ and PCO_2 were lower in S. Arterial K^+ and RR were the only parameters significantly different between S and NS at all time points during HH. Arterial levels of K^+ showed the clearest distinction between S and NS and may explain the sudden death experienced by animals during HH. The data suggest that early oxygenation and metabolic compensation are essential for survival of prolonged HH.

Keywords: rat, shock, potassium, cardiac output, oxygen delivery and consumption.

INTRODUCTION

Hemorrhagic shock involves the loss of a substantial portion of the circulating blood volume. The loss of volume causes decreases in cardiac output and hence oxygen (O_2) delivery to the peripheral tissues. Hypoperfusion to many tissues may be exacerbated by neuroendocrine reflexes that cause vasoconstriction. Nearly half of all patients suffering from hypovolemia with hemorrhagic shock die within the first 24 h. If untreated, hemorrhagic shock can lead to acidosis and cellular hypoxia, microcirculatory damage, multiple organ failure and ultimately death (13).

In marked hypotension and decreased tissue perfusion due to hypovolemic shock, correction of the initial problem may not correct the hypotension, because peripheral vasodilation has supervened: vasodilatory shock can follow volume resuscitation in prolonged and severe hypotension due to hemorrhage, known as "irreversible" or late-phase hemorrhagic shock (33). In addition, decreased systemic vascular resistance can be often found in less severe but prolonged hemorrhagic shock (19). Therefore, some studies have focused on the vasodilatory aspect of shock decompensation and have suggested therapeutic procedures for restoring vasoreactivity in order to reverse the vasodilation (19).

An alternative approach is to focus on the oxygenation differences. It has been repeatedly demonstrated in clinical and experimental shock that survival from hemorrhage is related in large part to the degree of developed O_2 debt and that, given the same hemorrhage insult, survival cannot be predicted based on volume loss or the resulting systemic or central hemodynamic variables such as blood pressure or cardiac output (10). The degree of O_2 debt developed during hemorrhage is highly predictive of the severity of subsequent reperfusion injury and downstream immune and inflammatory events (24).

The American College of Surgery defines shock as "an abnormality of the circulatory system that results in inadequate organ perfusion and tissue oxygenation"(1). Accordingly, many investigators consider that the measurement of perfusion-related variables such as O_2 deficit and base excess are better indicators of the severity of hemodynamic decompensation (10, 28).

In contrast, monitoring of shock is done most frequently by measuring mean arterial pressure, heart rate, central venous pressure, hematocrit or urine flow. These traditionally monitored variables may not adequately reflect tissue oxygenation and the severity of the cellular injury (28). Therefore, the careful selection of physiological parameters to be monitored is a critical step in the ability to distinguish between potential survivors and nonsurvivors to a prolonged hemorrhagic hypotension (HH) (10). It is important to simultaneously approach a variety of parameters in order to analyze the interactions of cardiac, pulmonary and tissue perfusion/oxygenation functions.

Few studies provide a rigorous examination of the relevance of each monitored variable during a prolonged hemorrhagic hypotension (7, 28). If a variable is unable to differentiate between survivors and nonsurvivors, it hardly qualifies as a useful clinical monitoring tool (28, 29). Therefore, this study was designed to provide a systematic investigation of physiological parameters that describe biochemical and O_2 transport patterns in animals subjected to hemorrhagic shock. Our hypothesis was that oxygenation differences play a major role in the ability of some animals to survive a prolonged HH with crystalloid alone to maintain blood pressure. An effort was made to identify which central hemodynamic, metabolic and systemic oxygenation parameters could differentiate survivors from nonsurvivors. An extensive analysis was performed in order to determine the systemic changes related not only to the response to HH, but also to the mechanisms behind the mortality in this model.

MATERIALS AND METHODS

Experimental animals. This study was approved in advance by the Institutional Animal Care and Use Committee of Virginia Commonwealth University Health System and conforms to the Public Health Service Policy on Human Care and Use of Laboratory Animals (August, 2002) and the American Physiological Society's Guiding Principles in the Care and Use of Animals. Twenty-one male Sprague-Dawley rats (Harlan, Indianapolis, IN) weighing 281 ± 9 g (mean ± SEM) were used in the study. Rats were housed in plastic cages placed in a facility with a 12 h light/dark cycle, constant temperature (21 – 23°C) and humidity (48 – 50%) and maintained for an adaptation period of one week. They were fed a commercial diet and had free access to water.

Aortic flow probe implantation. Rats were initially anesthetized with isoflurane (3%, balanced O_2, Abbott Laboratories, North Chicago, IL) then an oral intubation was performed in order to ventilate mechanically by a rodent pressure controlled ventilator (Kent Scientific, Torrington, CT). Under aseptic conditions, a thoracotomy was performed and the ascending aorta was isolated by blunt dissection and retraction of the thymus. A transit-time ultrasonic flow probe (model 2.5SB, Transonic Systems Inc., Ithaca, NY) was then positioned around the ascending aorta. The thorax was closed and acute experiments were carried out after a 6 – 9 day recovery period following the surgical procedure.

Hemodynamic measurements. The implanted animals were subjected to a tracheostomy and catheterizations under anesthesia with a mixture of Ketamine (70 mg/kg ip, Fort Dodge Animal Health, Fort Dodge, IA) and Acepromazine (3 mg/kg ip, Vedco, Inc., St. Joseph, MO), followed by constant intravenous infusion (0.24 – 0.36 mg/kg/h) of alfaxalone/alfadolone acetate (Saffan, Schering-Plough Animal Health, Welwyn Garden City, England). The left femoral vein was cannulated with PE-50 tubing for this purpose. The right carotid artery was cannulated with PE-

50 tubing filled with a heparin-saline solution (10 units heparin/ml), and connected to a pressure transducer to continuously measure arterial blood pressure (AP). The right jugular vein was cannulated with PE-90 tubing advanced to the entrance of the right atrium. This line was used to collect central venous blood samples and for recording central venous blood pressure (CVP). The left femoral artery was cannulated and connected to a microprocessor-controlled infusion/withdrawal syringe pump (model PHD2000, Harvard Apparatus, Holliston, MA). The same artery was used to hemorrhage the animal (see below) and to collect blood samples. All catheters were flushed as needed with heparinized saline to inhibit formation of clots. The core temperature was monitored and maintained at 36.5 – 37.0 °C using a thermostatically-controlled heating blanket (Harvard Apparatus, Holliston, MA). Normal saline was administered at room temperature (23 ± 2 °C) as hydration fluid during surgery (10 ml/kg i.p.).

The left spinotrapezius muscle was prepared for intravital microscopy first by making a dorsal midline incision extending approximately 5 cm caudally. The muscle was separated from the deep dorsal muscles and placed (ventral side up) on a heated platform at its physiological length via sutures along its lateral margins. Finally, the muscle was covered with a plastic film (Saran Wrap, Dow Corning, Midland, MI) to minimize desiccation and atmospheric gas exchange. Finally, the platform holding the animal and the exteriorized muscle was placed on a microscope stage and kept there for the remainder of the experiment. Observations on the muscle microcirculation will be reported separately.

Blood-gas, hematological, and biochemical measurements. Blood analyses were performed in paired arterial and venous samples (0.1 ml each) collected at various time points using heparinized glass capillary tubes (Clinitubes, D957G-70-100, Radiometer, Copenhagen, Denmark). All blood samples were immediately replaced by an equal volume of Ringer's L-

lactate (RL, Baxter, Deerfield, IL, USA). Blood glucose, potassium, chloride, sodium, calcium, lactate, bicarbonate, base excess (BE), PO_2, PCO_2 and pH were measured with a blood gas analyzer (ABL 725, Radiometer, Copenhagen, Denmark). Total hemoglobin (Hb) concentration and Hb O_2 saturation were measured with a multiwavelength CO-oximeter adjusted for the oxyhemoglobin dissociation curve of rat (OSM3, Radiometer, Copenhagen, Denmark).

Inclusion criteria. The following criteria had to be met by each animal prior to being enrolled in the experimental protocol: 1) full body weight recovery from the day of flow probe implantation, 2) baseline mean arterial pressure (MAP) above 85 mm Hg, 3) starting bicarbonate between 18 and 30 mmol/l, 4) arterial O_2 saturation above 90 % and 5) Hb concentration above 10 g/dl. This helped to minimize the effects of initial dehydration, excessive blood loss during surgical preparation, hemodilution due to excessive catheter flushing and accidental food/water deprivation prior to surgery. However, some animals that initially were included in the protocol showed unusually poor responses to hemorrhage and were also excluded from analysis as explained in the "Results" section.

Protocol. Animals were heparinized (260 units/kg b.w) and, after at least a 20-min stabilization period, baseline measurements were begun by obtaining blood samples for determination of arterial and venous blood gases and chemistry. During each blood collection, a time mark was placed in the computer recording of the systemic parameters in order to precisely match the data being collected. A second set of baseline measurements was performed 10 min later. Immediately following sampling for baseline, hypotension was induced by slow removal (0.3 – 0.5 ml/min) of blood over a 15 – 20 minute period until MAP reached 40 mm Hg. Small amounts of additional blood were withdrawn if needed to lower MAP to 40 mm Hg. Ringer's lactate infusion was used to maintain MAP if it fell below 40 mm Hg. The amount of RL

infused was measured and did not exceed more than three times the shed blood volume. Systemic blood gases and metabolic parameters, along with systemic hemodynamics, were measured when the 40 mm Hg target was reached (considered as time point t = 0) and at one-half, one, two, three and four hours later. All animals surviving the entire 4-hour experiment, as well as those requiring early sacrifice, received a pentobarbital overdose of 100 mg/kg (i.v.). Control animals were subjected to all surgical and experimental procedures described above except for the controlled hemorrhage.

Data acquisition and analysis. The amplified outputs from the pressure transducers (DA100C, Biopac Systems, Goleta, CA), and those from the aortic flowmeter were connected to a desktop computer for continuous on-line data acquisition at a rate of 500 Hz (Acqknowledge 3.7.2 + MP150 hardware and software; Biopac Systems, Goleta, CA). The inspiratory phase of each respiratory cycle generated negative pressure changes in the CVP tracings, easily distinguishable from right atrial pressure changes. Respiratory rate was calculated as the reciprocal of the interval between these successive negative pressure peaks in the CVP tracings. Systolic, diastolic, pulse and mean arterial pressures (MAP) were calculated from the original digitized traces of AP. Heart rate (HR) was calculated from the aortic flow signal as the reciprocal of the interval between successive flow peaks and recorded continuously. Cardiac output (CO) was estimated from the mean aortic flow. Mean stroke volume (SV) was calculated as CO/HR. Cardiac index (CI) and stroke index (SI) were computed by dividing the appropriate variables by body mass. Total peripheral resistance (TPR) was calculated as (MAP – CVP) / CI. All off-line calculations were based on one-min segments of the digitized signals, taken as close as possible to the blood collection time points. Global oxygen consumption (VO_2) was calculated using the Fick principle as the product of cardiac index and the difference between arterial (CaO_2) and venous (CvO_2) O_2 contents:

$CaO_2 = (Hb \cdot 1.34 \cdot SaO_2) + (0.003 \cdot PaO_2)$; $CvO_2 = (Hb \cdot 1.34 \cdot SvO_2) + (0.003 \cdot PvO_2)$

$VO_2 = CI \cdot (CaO_2 - CvO_2)$

in which SaO_2 and SvO_2 are the arterial and venous O_2 saturations, respectively, and PaO_2 and PvO_2 are the arterial and venous O_2 tensions, respectively. Whole animal oxygen delivery (DO_2) was computed as the product of CaO_2 and CI. The O_2 extraction ratio (O_2ER) was computed as VO_2 / DO_2. VO_2 deficit was calculated as the difference between VO_2 during hypotension and the mean VO_2 during baseline measurements. The net cumulative O_2 debt at each time point was calculated from the integrated area described by the time-VO_2 deficit curve.

Statistics. Values are reported as mean ± SEM. Differences among groups were analyzed by using 2-way ANOVA with repeated measures. When a significant F-value was encoutered, post-hoc analyses were performed between groups using the Student-Newman-Keuls test. A level of $p<0.05$ was considered significant.

RESULTS

Control animals. Animals subjected to all procedures except hemorrhagic hypotension (HH) showed relatively stable physiological parameters throughout the experimental period of five hours. **Tables 1-3** present the average values of most systemic parameters for these animals. The steady decrease in Hb over time (also shown in **Figure 1**, right panel) was probably due to the small hemodilution consequent to successive blood sampling for gas/chemistry analyses. However, the animals maintained high O_2 saturation and PO_2, with stable DO_2 and VO_2. The remaining parameters for the control animals are presented in conjunction with the description of the results from animals subjected to HH.

Hemorrhaged animals. Some animals subjected to HH appeared to decompensate quite early and died less than 1 hour following blood withdrawal. This response could be due to transient falls to 30 or 35 mm Hg (e.g., caused by poor handling of bleeding procedure or failure to maintain MAP) that would significantly accelerate the time of decompensation. The early decompensation could also potentially indicate that the animals had not fully recovered from the previous surgery (i.e., flow probe implantation). Therefore, these animals were not included in the present analysis. The remaining animals subjected to HH (n=17) were divided into 2 groups according to survival time: 9 rats survived 3-4 hours (S, survivors) and 8 animals died in less than 3 hours after MAP reached 40 mm Hg (NS, nonsurvivors). Both groups of animals required similar bleed volumes to reach a MAP of 40 mm Hg: 33 ± 4 ml/kg and 28 ± 2 ml/kg for NS and S, respectively (**Figure 1**, left panel). The mean hemorrhage time to reach the MAP level of 40 mm Hg was also similar: 17.4 ± 1.3 min and 18.7 ± 1.0 min for NS and S, respectively. The mean time from MAP = 40 mm Hg to death was 129 ± 12 min and 220 ± 12 min for NS and S, respectively. All animals required crystalloid (Ringer's lactate) infusion to maintain MAP at 40 mm Hg for one hour or longer, but the amount of infused RL was significantly higher for NS

than S (**Figure 1**, left panel). The rate of RL infusion was also significantly higher for NS at t = 60 min (0.40 ± 0.04 ml/kg/min vs. 0.23 ± 0.06 ml/min kg) and at t = 120 min (0.43 ± 0.04 ml/kg/min vs. 0.27 ± 0.07 ml/min kg). Consequently, Hb concentration was lower for NS at all points during hypotension although differences did not reach statistical significance (**Figure 1**, right panel). One of the possible causes for the higher demand for RL infusion among NS could be the changes in total peripheral resistance (**Figure 2**). Immediately after 40 mm Hg was reached, survivors showed a larger increase in TPR (from baseline values) than NS (44.6 ± 11.3 vs. 21.7 ± 8.9 %, respectively, p=0.05). Although values only reached the significance level at t = 120 min, S showed higher TPR than NS during the whole hypotensive period (**Figure 2**).

Respiratory, hemodynamic and oxygenation responses. Hemodynamic and cardiac data from hemorrhaged animals are shown in **Tables 4 and 5**. Survivors showed a tendency to higher SI (p = 0.07) and lower HR (p = 0.12) than NS rats at baseline. Arterial blood pressures and cardiac parameters were similar for both groups of animals during HH except at the last time point for NS (120 min) where stroke index was higher in NS, probably reflecting an increased venous return due to the larger volume of infused RL. A lower heart rate was also observed at t = 120 min for NS animals. In contrast with the similar response of the cardiovascular parameters between S and NS, respiratory rate was systematically higher for S animals, from the start of the hypotensive period throughout the observation time (**Figure 3 A**). Biochemical values of arterial blood reflected a stronger respiratory response from S animals: PO_2, Hb O_2 saturation and O_2 content were higher in S, especially after the first hour of hypotension (**Figure 3 B-D**). Similar values were found for venous blood.

The development of lactic acidosis with respiratory compensation is further characterized by the data shown in **Figures 4 and 5**. Hemorrhaged animals exhibited decreased pH, PCO_2, BE and HCO_3^- with simultaneous increase in lactate. Changes in HCO_3^-, BE and lactate were similar for NS and S animals despite larger the volume received in NS (**Figure 4**). The survivor group showed better compensatory responses as expressed by higher levels of PCO_2 and pH (**Figure 5**). Again, similar values were found for venous blood.

Table 6 presents data on whole body O_2 delivery (DO_2), O_2 consumption (VO_2) and O_2 extraction ratio (O_2ER). **Figure 6** illustrates the relationship between VO_2 and DO_2 (averaged over seven DO_2 ranges) for both groups of animals. A clear region of DO_2-dependent O_2 consumption was present. The inflection in the VO_2-DO_2 relationship was similar for both groups of rats. Although similar values during hypotension were achieved by NS and S animals, the data suggest that the responses were actually different since animals from the survivor group during baseline had lower O_2ER ($p = 0.06$) and a slightly lower VO_2 ($p = 0.32$) than NS rats. Therefore, during the first 2 h of HH, the survivors showed tendencies to a smaller average drop (from baseline) in VO_2 (35 ± 2 % vs. 42 ± 3 %, $p = 0.09$) and to a larger average increase in O_2ER (73 ± 12 % vs. 32 ± 11 %, $p = 0.20$). Since even small differences in VO_2 may be significant over time, the VO_2 deficit was used to estimate O_2 debt and the cumulative O_2 debt (**Figure 7**). Although the variability of the data prevented the demonstration of statistical significance at all time points, the data shown in **Figure 7** are suggestive that the small difference in VO_2 deficit between NS and S translated into a larger O_2 debt for NS animals ($0.1 > p > 0.05$ for O_2 debt and for cumulative O_2 debt at $t = 120$ min) .

Blood electrolytes and glucose. Table 7 shows data on changes in plasmatic Na^+, Cl^- and Ca^{++}. Transient hyperchloremia and progressive hypocalcemia (15% decrease from baseline, $0.1 > p > 0.05$ at $t = 120$ min) were observed during HH, whereas blood Na^+ levels did not vary significantly over time. All these changes followed similar patterns in NS and S animals.

In spite of the variability in oxygenation responses for NS and S animals, the pattern of death was similar for all rats. Even though various levels of systemic parameters were found, all animals appeared to die in a similar and abrupt manner. As illustrated in **Figure 8**, there was typically an irreversible and rapid fall in arterial pressure, heart rate and aortic flow, suggestive of an acute cardiac event. A potential candidate for such an effect is the intravascular potassium level. In fact, changes in plasma K^+ levels followed a very distinctive pattern as illustrated in **Figure 9 (panels A and B)**. At all time points during hypotension, NS had a higher K^+ level than that of S animals, the difference ranging from 1 to 3 mmol/l. However, the K^+ levels at death were very high (above 9 mmol/l) and similar between S and NS. Arterial glucose levels (**Figure 9 C**) were also significantly different between S and NS, survivors showing less pronounced hypoglycemia than NS after the hyperglycemic response to acute blood withdrawal.

DISCUSSION

The main findings in this study were that 1) survivors showed better oxygenation responses to HH than nonsurvivors as supported by higher arterial PO_2, arterial and venous Hb O_2 saturation and O_2 content; 2) better ventilation seems to explain these findings, since survivors showed higher respiratory rate than NS throughout the HH; 3) S and NS animals died during HH under high plasma K^+ and lactate, together with low levels of glucose, Ca^{++}, BE, HCO_3^- and pH; 4) survivors showed a tendency to lower O_2 debt (nearly significant; $0.1 > p > 0.05$) than nonsurvivors; 5) the rate and amount of infused RL necessary to keep MAP at 40 mm Hg was higher in NS. Among all parameters investigated, arterial K^+ and ventilatory frequency were the only parameters significantly different between S and NS at all time points during HH, suggesting that early oxygenation and metabolic compensation are essential for the survival to prolonged HH.

We developed a stable preparation that did not suffer deterioration in central hemodynamics during the five hour experimental period. In addition, the mortality in animals experiencing hemorrhagic shock was approximately 50% within 2 hours. This produced a clinically relevant model as recommended by the Committee On Fluid Resuscitation For Combat Casualties (23), and provided sufficient numbers of surviving animals to allow statistical comparisons between survivors and nonsurvivors. Over 50 different systemic parameters were investigated in hemorrhaged animals in an effort to identify the most discriminating ones between survivors and nonsurvivors. Several of these measurements were different between survivors and nonsurvivors, and possibly of prognostic value in shock, especially since these measurements are already clinically available. A protocol of prolonged (up to 4 hours) HH was chosen because delayed interventions in hemorrhaged subjects may occur in both civilian and military scenarios,

where resuscitation resources (crystalloids) are scarse and blood products are normally not available (9). However, there are relatively few studies examining the respiratory, cardiovascular and metabolic consequences of prolonged HH (MAP of 40 mm Hg). Such a time and severity combination is probably uniformly lethal in rats. Johnson et al (15) found that the average time from initiation of hemorrhage to a MAP of 40 mm Hg until death in a Wiggers' model was just under three hours. Hemorrhage to 40 mm Hg for only one hour followed by resuscitation was lethal to approximately 2/3 of rats (42). We have observed a mortality of 82% during four hours of shock. However, some species are more tolerant of such an insult: 68% of conscious hamsters survived four hours of shock at a MAP of 40 mm Hg (17).

Although it has been shown that preheparinization (2000 U/kg) has protective effects in hemorrhaged rats (38), it is unlikely that the single dose used in the present experiments (260 U/kg) affected the responses (41). In addition, the infusion of RL to maintain a MAP of 40 mm Hg would have diluted even further the heparin given before baseline measurements. The anesthetic (Saffan) was chosen because it preserves both the interaction between injury and cardiovascular reflex activity and also, the defense reactions (20). Injury-induced changes in baroreflex sensitivity under Saffan anesthesia are similar to those seen in conscious humans (20). Several studies on cardiovascular and respiratory functions in the rat have been performed using this anesthetic (34), including studies involving determinations of DO_2 and VO_2 (11). Since CO cannot be accurately estimated from arterial pressure and heart rate (39), we continuously measured CO. In addition, direct measurement of CO was important for estimate of TPR, VO_2 and DO_2.

Vasoconstriction in the peripheral circulation is the normal immediate response to conditions in which the arterial pressure is too low for adequate tissue perfusion, such as HH. Delayed hypotension may occur as a result of failure of the vascular smooth muscle to constrict. Such so-called vasodilatory shock is characterized not only by hypotension due to peripheral vasodilation, but also by a poor response to therapy with vasopressor drugs. Our data are consistent with findings of a vasodilatory shock that follows volume resuscitation during prolonged and severe hypotension due to hemorrhage (33). Hemorrhaged animals in this study showed only a transient increase in TPR (more pronounced for S) and differences in TPR during HH between S and NS corresponded to different rates of RL infusion. However, the different rates of RL infusion could also reflect differences in the venous capacitance between S and NS.

The vasodilatory shock can be the final common pathway for long-lasting and severe shock of any cause. In marked hypotension and decreased tissue perfusion due to HH, correction of the initial problem may not correct the hypotension, because peripheral vasodilation has supervened (19). Vasodilation found during the decompensatory phase of hemorrhagic shock, such as the one found in the present experiments, has been associated with K^+ channels (19). Potassium channels closed by increases in intracellular ATP levels (K^+-ATP channels) have been described in vascular smooth muscle cells and other cell types. These channels can be opened by a decrease in intracellular ATP levels and intra- or extracellular acidosis. Hemorrhagic shock is associated with early vasomotor paralysis as well as with early derangements in the intracellular metabolic status (5). Activation of K^+-ATP channels has been shown to contribute to the vasodilation and early mortality in a rat model of severe hemorrhagic shock. In anesthetized rats

hemorrhaged to a MAP of 35 mmHg, inhibition of K^+-ATP channels with glibenclamide or tolazamide increased MAP and improved survival rate (31).

In addition, systemic pressor responsiveness and arterial reactivity to angiotensin II are selectively impaired at an early stage of HH. This phenomenon partially involves nitric oxide and is not related to ATP-sensitive K^+ channels (22). Nitric oxide has also been reported to increase in response to HH (30), and may modulate at least a portion of the observed changes in VO_2 during HH (26). Several other mediators have been considered in the acidosis-induced dilation including histamine, prostanoids, electrogenic Na^+ pump and newly synthesized cytochrome P450 metabolites (8, 15).

While vasodilation is an important component of irreversible shock, it may not have been the cause of death observed in the present experiments. Although hyperkalemia has not been directly associated with death following HH in rats, our data are suggestive that this may be the case. The same maximum levels of K^+ found in survivors and in nonsurvivors in our study (8 to 10 mmol/l) have been previously found at death in rats (16, 21, 36). Considering the hepatic K^+ content of 100 μmol/g (16), ischemia-induced loss of hepatic K^+ could account for a portion of the observed increase in extracellular K^+. This is also supported by the fact that the liver during shock has been shown to experience large decreases in blood flow (25), which are not reversed even with RL resuscitation (37), and the most severe reduction in VO_2 associated with an unchanged O_2ER (2). We suggest that acute cardiorespiratory failure leading to death during prolonged HH is due to severe hyperkalemia. The overall decline in cardiac function during HH

can be partially explained by a number of additional findings in this study such as decreased Ca^{++}, glucose, pH, PO_2 and Hb O_2 saturation. Other studies have shown that increased levels of TNF-alpha (3) and lower ATP levels also contribute to cardiac depression (as well as immunological responses) during HH in rats (5, 12, 18).

Among all parameters investigated, arterial K^+ was the parameter that showed the clearest distinction between S and NS at all time points during HH. The rate of rise in plasma K^+ was remarkably different between the two groups of animals. The finding of higher levels of K^+ in NS is particularly significant considering that major differences were found in the amount of infused RL between NS and S. NS animals appeared to have higher release of intracellular K^+ since the levels of plasmatic K^+ were diluted by the large amount of infused RL. For instance, at t = 120 min, the plasmatic concentration of K^+ in NS was 50 % higher than S (**Figure 9A**) in spite of a 67 % higher amount of infused RL (**Figure 1, left**). Elevated levels of K^+ have been found previously during HH (16) but, in most cases, K^+ has been associated with vasodilation, as discussed above. Recent evidence supports the concept that treatments (hypothermia) leading to decreasing levels of hyperkalemia during HH improve outcome (16, 40). In addition, hypoglycemia has been found in our animals during prolonged HH. Insulin and glucose have been suggested in the treatment of HH leading to increased hepatic ATP (4). Although these regimens provoke hypokalemia, their beneficial role should be counterbalanced with the hypoglycemic effect.

The most striking difference between S and NS was the ability of S to systemically oxygenate better than nonsurvivors. This would favor the idea of oxygen therapy in HH. However, O_2 therapy to increase arterial PO_2 should be used with caution since O_2 breathing has improved the outcome in some studies with rats (6) but failed to do so in others (32). Differences in the severity of the HH, anesthetic agent and in the achieved level of arterial PO_2 are probably involved in these discrepancies. In addition, beneficial actions of hyperoxia should be counterbalanced by its microvascular effects such as vasoconstriction and decreased functional capillary density (35). Nevertheless, our data support the concept of a "physiological" increase in oxygenation since survivor animals were breathing normal FiO_2 and achieved higher PO_2, Hb O_2 saturation and O_2 content by increased ventilatory rate and by a larger average increase in O_2 ER.

Bulk movement of O_2 is a useful measure of tissue perfusion because 1) it can be measured using arterial and mixed venous blood; 2) it has a large arteriovenous gradient; and 3) it is related to overall tissue perfusion and outcome. In previous studies, overall O_2 delivery (DO_2) has been measured by the product of CO and arterial O_2 content, whereas overall body metabolism was evaluated by O_2 consumption (VO_2) using the product of CO and the arteriovenous O_2 content difference (25, 26). In the current study, whole-body DO_2 and VO_2 were also measured using this approach. A well-defined, delivery-dependent portion of the DO_2 vs. VO_2 relationship was found when values obtained during HH were computed. All hemorrhaged animals remained at

this delivery-dependent stage throughout the HH period. All animals fell bellow critical DO_2 when the target MAP was reached. The value of critical DO_2 was similar for S and NS.

The degree and duration of tissue hypoxia have been estimated by calculating the O_2 debt from the difference between VO_2 before and during hemorrhage. A similar procedure has been used previously (27). In our study, a consistent pattern of smaller O_2 debt was found for S during HH. This is compatible with a better oxygenation response by the survivors. The correlation between poor oxygenation (and increased O_2 debt) and outcome in HH has been documented in rats, pigs, dogs and humans (10, 27, 29). Somewhat similar results were found in hamsters in a study that also examined the microcirculation of skin (17).

An interesting observation was that traditional metabolic indices of shock severity such as lactate, BE and HCO_3^- were not different between S and NS. This contrasts with some reports in humans (10), but it is consistent with the view that lactate may be of limited value as an indicator of tissue hypoxia (14). In addition, cardiac index was not significantly different between survivors and nonsurvivors. Similar findings have been reported in humans (28). However, it should be noted that, during baseline, survivors had a tendency to show lower HR and higher SI than nonsurvivors. This finding may be significant since it suggests that better resting cardiac conditioning (traditionally expressed by low HR and high SI) may be an additional beneficial factor for the survival of prolonged HH.

In summary, the present studies in hemorrhaged rats document distinct patterns in survivors and

nonsurvivors. The main features of the early cardiorespiratory and metabolic patterns of the animals that died in less than 3 hours included lower values for respiratory rate, blood PO_2, pH, BE and HCO_3^-. Differences between the two groups in most physiologic variables were not dramatic (and did not reach statistical significance), but the overall pattern of changes was consistent. Non-survivors showed a significantly greater degree of metabolic acidosis than survivors following the onset of hemorrhage. Arterial K^+ was the parameter that showed the clearest distinction between S and NS at all time points and the elevated levels of K^+ may explain the sudden death experienced by animals during HH. The data suggest that early oxygenation and metabolic compensation are essential for the survival of prolonged HH.

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FIGURE LEGENDS

Figure 1. Left panel. Total volume of hemorrhaged blood (symbols, at left) followed by cumulative volume of Ringer's lactate solution infused to maintain MAP at 40 mm Hg. **Right panel.** Venous hemoglobin concentration at various time points before and during hemorrhagic hypotension. Data points shown at BL (baseline) are measurements performed before hemorrhage. Time = 0 min means the 40 mm Hg target pressure was reached. ■ = control animals (n=4), ▽ = animals surviving less than 3 h of HH (nonsurvivors, n=8), ○ = rats surviving 3-4 h (survivors, n=9). † significantly different from baseline period; ‡ significantly different from control group at same time point; * significantly different from survivors at same time point. All data expressed as mean ± SEM.

Figure 2. Total peripheral resistance for all studied animals in percent of change from control. Survivors showed higher TPR at t = 0 min ($p = 0.05$) and t = 30 min ($p = 0.13$). Data shown as in **Figure 1**.

Figure 3. Panel A. Respiratory rate for all studied animals. **Panels B-D.** Arterial Hb O_2 saturation, PO_2 and O_2 content for all animals. Note increase in Hb O_2 saturation and PO_2 soon after blood withdrawal at t = 0 min. Data shown as in **Figure 1**.

Figure 4. Arterial blood levels of base excess, HCO_3^- and lactate. Data shown as in **Figure 1**.

Figure 5. Arterial blood levels of pH and PCO_2. Data shown as in **Figure 1**.

Figure 6. Systemic VO_2 as a function of systemic DO_2 for hemorrhaged animals. Each point represents the average of at least 5 determinations of VO_2 at a given range of DO_2. Error bars represent SEM.

Figure 7. Panel A. Difference between mean calculated VO_2 at each time point and mean baseline VO_2. **Panels B and C.** O_2 debt and the cumulative O_2 debt for all studied animals. The O_2 debt and the cumulative O_2 debt were larger for NS animals at t = 120 min ($0.1 > p > 0.05$). Data shown as in **Figure 1**.

Figure 8. Original tracings of a typical experiment illustrating the rapid death experienced by animals during prolonged hemorrhagic hypotension. From top to bottom: mean arterial pressure, central venous pressure, aortic blood flow, mean arterial blood pressure and mean aortic flow.

Figure 9. Arterial (panel A) and venous (panel B) plasma potassium levels. At all time points during hypotension, survivors had a significantly lower potassium level than that of NS animals. K^+ levels at t = 120 min for NS and at t = 240 for S animals are not significantly different. Arterial (panel C) and venous (panel D) glucose levels. Note hyperglycemic response to acute blood withdrawal at t=0 min. Data shown as in **Figure 1**.

Table 1. **Cardiovascular parameters of control (normovolemic) animals.**

Parameter	Sham Hemorrhagic Hypotension						
	Baseline	**0 min**	**30 min**	**60 min**	**120 min**	**180 min**	**240 min**
MAP (mmHg)	101.0 ± 6.5	106.3 ± 7.2	104.5 ± 6.0	102.6 ± 8.6	105.7 ± 4.7	104.9 ± 0.8	97.3 ± 8.1
DP (mmHg)	75.3 ± 5.1	77.1 ± 5.2	78.2 ± 7.0	74.1 ± 8.4	72.8 ± 8.9	74.6 ± 3.9	63.4 ± 6.1
SP (mmHg)	130.4 ± 8.2	138.1 ± 10.2	133.3 ± 7.6	134.2 ± 10.9	142.0 ± 2.3	143.4 ± 2.2	140.8 ± 8.7
PP (mmHg)	55.1 ± 5.8	61.0 ± 6.0	55.1 ± 6.4	60.1 ± 9.7	69.2 ± 7.0	68.8 ± 6.1	77.4 ± 2.8†
CVP (mmHg)	0.3 ± 0.4	0.2 ± 0.4	0.4 ± 0.4	0.2 ± 0.3	0.2 ± 0.3	0.3 ± 0.4	0.7 ± 0.3
TPR (mmHg/ml/min)	0.60 ± 0.05	0.62 ± 0.06	0.65 ± 0.08	0.63 ± 0.08	0.56 ± 0.07	0.54 ± 0.07	0.49 ± 0.05
CI (ml/min/kg)	170.8 ± 15.7	174.3 ± 17.1	166.9 ± 17.6	169.6 ± 23.9	193.2 ± 13.3	202.0 ± 23.1	205.1 ± 34.2
SI (µl/kg)	456.7 ± 20.5	457.9 ± 25.6	435.8 ± 34.0	445.6 ± 44.7	485.7 ± 20.2	497.0 ± 39.9	504.9 ± 59.8
HR (min^{-1})	401.0 ± 13.6	391.1 ± 12.6	376.5 ± 14.4	373.1 ± 18.6	396.8 ± 11.4	404.0 ± 15.4	400.8 ± 22.8

Values are means ± SEM from 4 rats. MAP, mean arterial pressure; DP, diastolic pressure; SP, systolic pressure; PP, pulse pressure; CVP, central venous pressure; TPR, total peripheral resistance; CI, cardiac index; SI, stroke index; HR, heart rate.

† significantly different from baseline period.

Table 2. **Blood biochemical parameters of the control (normovolemic) animals.**

Parameter	Sham Hemorrhagic Hypotension						
	Baseline	**0 min**	**30 min**	**60 min**	**120 min**	**180 min**	**240 min**
a Na^+ (mmol/l)	139.8 ± 1.3	140.3 ± 1.3	140.3 ± 1.3	140.3 ± 1.3	140.3 ± 1.7	142.0 ± 3.5	141.5 ± 3.2
a Ca^{++} (mmol/l)	2.56 ± 0.04	2.55 ± 0.04	2.53 ± 0.03	2.53 ± 0.02	2.48 ± 0.03	2.54 ± 0.06	2.52 ± 0.05
a Cl^- (mmol/l)	106.8 ± 0.7	106.8 ± 0.8	107.0 ± 0.7	107.0 ± 0.9	106.3 ± 0.9	105.5 ± 1.0	106.0 ± 1.1
v Na^+ (mmol/l)	141.5 ± 1.1	142.3 ± 1.8	143.3 ± 0.9	143.5 ± 1.3	142.3 ± 1.8	144.8 ± 2.6	143.0 ± 3.2
v Ca^{++} (mmol/l)	2.50 ± 0.05	2.43 ± 0.05	2.45 ± 0.07	2.45 ± 0.06	2.45 ± 0.04	2.41 ± 0.10	2.51 ± 0.07
v Cl^- (mmol/l)	107.5 ± 0.8	109.5 ± 1.7	111.0 ± 1.6	110.3 ± 2.0	107.3 ± 1.5	109.0 ± 1.5	105.3 ± 0.6
v pH	7.41 ± 0.02	7.43 ± 0.02	7.40 ± 0.01	7.41 ± 0.01	7.42 ± 0.01	7.42 ± 0.01	7.45 ± 0.01
v PCO_2 (mmHg)	39.7 ± 1.4	37.0 ± 1.6	37.0 ± 1.6	37.4 ± 1.9	38.9 ± 2.5	37.1 ± 1.2	36.5 ± 0.3
v Lactate (mmol/l)	1.1 ± 0.1	0.9 ± 0.1	1.4 ± 0.2	1.4 ± 0.2	2.4 ± 0.7	2.5 ± 0.9	3.5 ± 1.5
v BE (mmol/l)	0.5 ± 1.1	0.0 ± 1.0	-1.5 ± 0.6	-0.8 ± 1.5	0.2 ± 1.2	-1.6 ± 1.8	-1.5 ± 2.7
v HCO_3 (mmol/l)	24.4 ± 0.9	24.2 ± 0.9	23.0 ± 0.4	23.5 ± 1.1	24.4 ± 0.9	23.0 ± 1.5	22.9 ± 2.4

Values are means ± SEM from 4 rats. a, arterial; v, venous; BE, base excess.

Table 3. **Oxygenation parameters of the control (normovolemic) animals.**

Parameter	Sham Hemorrhagic Hypotension						
	Baseline	**0 min**	**30 min**	**60 min**	**120 min**	**180 min**	**240 min**
v Hb (g/dl)	12.3 ± 0.7	11.8 ± 0.7	11.7 ± 0.6	11.3 ± 0.5	10.8 ± 0.6	10.1 ± 0.4†	9.7 ± 0.5†
v O_2 ct (ml%)	10.8 ± 1.2	10.4 ± 1.2	10.6 ± 0.7	10.0 ± 0.6	9.8 ± 0.8	9.1 ± 0.4	8.8 ± 0.8
v SO_2 (%)	63.7 ± 5.6	64.0 ± 5.6	66.3 ± 3.4	64.4 ± 2.6	66.4 ± 4.2	63.9 ± 3.8	63.7 ± 7.6
v PO_2 (mmHg)	43.0 ± 4.7	41.8 ± 3.0	45.3 ± 3.5	44.9 ± 5.1	45.2 ± 3.6	45.2 ± 6.1	43.2 ± 6.6
DO_2 (ml/min/kg)	26.6 ± 1.0	26.8 ± 1.3	25.2 ± 1.4	25.4 ± 2.4	27.2 ± 0.4	27.1 ± 1.7	27.0 ± 3.8
VO_2 (ml/min/kg)	9.0 ± 1.4	9.5 ± 1.6	8.2 ± 0.7	9.1 ± 1.0	8.9 ± 1.2	9.3 ± 1.0	9.1 ± 1.7
O_2 ER	0.34 ± 0.05	0.35 ± 0.06	0.33 ± 0.03	0.36 ± 0.03	0.33 ± 0.04	0.34 ± 0.04	0.35 ± 0.07

Values are means ± SEM from 4 rats. v, venous; Hb, hemoglobin concentration; O_2 ct, oxygen content; SO_2, oxygen saturation; PO_2, oxygen partial pressure; DO_2, systemic oxygen delivery; VO_2, systemic oxygen consumption; O_2 ER, oxygen extraction ratio. † significantly different from baseline period.

Table 4. **Hemodynamic parameters during prolonged hemorrhagic hypotension.**

Parameter	Group	Baseline	Hemorrhagic Hypotension					
			0 min	30 min	60 min	120 min	180 min	240 min
MAP (mmHg)	S	101.9 ± 4.1	41.4 ± 1.0†‡	37.7 ± 1.1†‡	38.6 ± 0.9†‡	39.8 ± 0.3†‡	38.5 ± 1.2†‡	36.1 ± 3.1†‡
	NS	110.8 ± 4.9	41.4 ± 0.7†‡	40.1 ± 0.4†‡	40.3 ± 0.4†‡	40.3 ± 0.7†‡		
DP (mmHg)	S	78.6 ± 5.1	26.4 ± 2.7†‡	24.1 ± 1.7†‡	24.2 ± 1.1†‡	23.6 ± 1.2†‡	20.4 ± 2.3†‡	19.0 ± 2.5†‡
	NS	90.9 ± 5.8	28.8 ± 0.7†‡	26.5 ± 1.3†‡	25.8 ± 2.0†‡	22.3 ± 1.6†‡		
SP (mmHg)	S	128.7 ± 4.9	71.8 ± 6.6†‡	66.5 ± 4.6†‡	71.2 ± 8.5†‡	79.4 ± 8.8†‡	86.7 ± 7.6†‡	99.1 ± 9.0
	NS	130.5 ± 4.5	59.6 ± 6.2†‡	63.1 ± 7.6†‡	69.3 ± 9.7†‡	83.8 ± 11.8†‡		
PP (mmHg)	S	50.1 ± 5.2	42.6 ± 7.7	42.4 ± 5.8	53.6 ± 8.0	62.3 ± 8.9	66.2 ± 8.8	80.1 ± 9.2†
	NS	39.6 ± 3.9	30.8 ± 6.4†‡	36.6 ± 8.6	43.4 ± 11.5	61.5 ± 13.2		
CVP (mmHg)	S	0.7 ± 0.3	-1.3 ± 0.4	-0.3 ± 0.6	-0.6 ± 0.4	-0.1 ± 0.3	0.3 ± 0.3	1.6 ± 0.5
	NS	-0.3 ± 0.5	-2.2 ± 0.3	-1.9 ± 0.3†‡*	-1.4 ± 0.4†‡	-0.2 ± 0.5		
TPR (mmHg/ml/min)	S	0.47 ± 0.02	0.67 ± 0.04†	0.66 ± 0.04†	0.55 ± 0.04	0.42 ± 0.02‡	0.39 ± 0.03	0.40 ± 0.07
	NS	0.61 ± 0.07	0.72 ± 0.08	0.68 ± 0.09	0.55 ± 0.06	0.42 ± 0.05		

Values are means ± SEM from 9 survivors (S) and 8 nonsurvivors (NS).

MAP, mean arterial pressure; DP, diastolic pressure; SP, systolic pressure;

PP, pulse pressure; CVP, central venous pressure; TPR, total peripheral resistance.

† significantly different from baseline period;

‡ significantly different from control group at same time point;

* significantly different from S group.

Table 5. **Cardiac parameters during prolonged hemorrhagic hypotension.**

Parameter	Group	Baseline	Hemorrhagic Hypotension					
			0 min	30 min	60 min	120 min	180 min	240 min
CI (ml/min/kg)	S	217.2 ± 8.9	66.1 ± 5.3†‡	60.5 ± 6.3†‡	75.0 ± 6.3†‡	96.1 ± 4.3†‡	101.1 ± 5.7†‡	94.8 ± 19.7†‡
	NS	196.9 ± 20.0	64.4 ± 7.4†‡	67.2 ± 7.7†‡	79.0 ± 6.7†‡	100.5 ± 11.4†‡		
SI (µl/kg)	S	568.4 ± 24.4	188.1 ± 14.8†‡	164.1 ± 14.2†‡	219.3 ± 18.6†‡	293.0 ± 21.9†‡	349.3 ± 26.0†‡	379.6 ± 61.9
	NS	475.2 ± 42.9	158.7 ± 14.6†‡	179.3 ± 20.9†‡	227.3 ± 20.9†‡	398.6 ± 43.2*		
HR (min^{-1})	S	383.3 ± 9.6	354.9 ± 15.1	366.1 ± 16.1	346.1 ± 19.4	335.4 ± 17.2	297.5 ± 20.7†‡	249.2 ± 39.5†‡
	NS	411.4 ± 8.6	400.9 ± 11.1	376.9 ± 14.6	351.0 ± 17.0	264.2 ± 31.6†‡*		

Values are means ± SEM from 9 survivors (S) and 8 nonsurvivors (NS).

CI, cardiac index; SI, stroke index; HR, heart rate.

† significantly different from baseline period;

‡ significantly different from control group at same time point;

* significantly different from S group.

Table 6. **Oxygenation parameters during prolonged hemorrhagic hypotension.**

Parameter	Group	Baseline	Hemorrhagic Hypotension					
			0 min	30 min	60 min	120 min	180 min	240 min
DO_2 (ml/min/kg)	S	34.4 ± 2.0	8.1 ± 0.9†‡	7.2 ± 0.6†‡	8.3 ± 0.6†‡	9.8 ± 0.5†‡	8.6 ± 0.8†‡	8.1 ± 1.7†‡
	NS	30.9 ± 3.8	7.5 ± 0.8†‡	7.5 ± 0.8†‡	7.8 ± 0.7†‡	7.9 ± 1.3†‡		
VO_2 (ml/min/kg)	S	9.3 ± 0.9	5.9 ± 0.7	5.6 ± 0.4†‡	5.6 ± 0.5†‡	6.7 ± 0.5	5.5 ± 0.6†‡	4.5 ± 0.6†‡
	NS	10.7 ± 1.1	6.2 ± 0.8	6.1 ± 0.8†‡	5.4 ± 1.0†‡	6.4 ± 1.2		
O_2 ER	S	0.27 ± 0.03†‡	0.74 ± 0.03†‡	0.79 ± 0.04†‡	0.69 ± 0.06†‡	0.68 ± 0.05†‡	0.64 ± 0.05‡	0.58 ± 0.07
	NS	0.37 ± 0.04†‡	0.82 ± 0.03†‡	0.79 ± 0.03†‡	0.78 ± 0.04†‡	0.81 ± 0.01†‡*		

Values are means ± SEM from 9 survivors (S) and 8 nonsurvivors (NS).

DO_2, systemic oxygen delivery; VO_2, systemic oxygen consumption;

O_2 ER, oxygen extraction ratio.

† significantly different from baseline period;

‡ significantly different from control group at same time point,

* significantly different from S group.

Figure 1

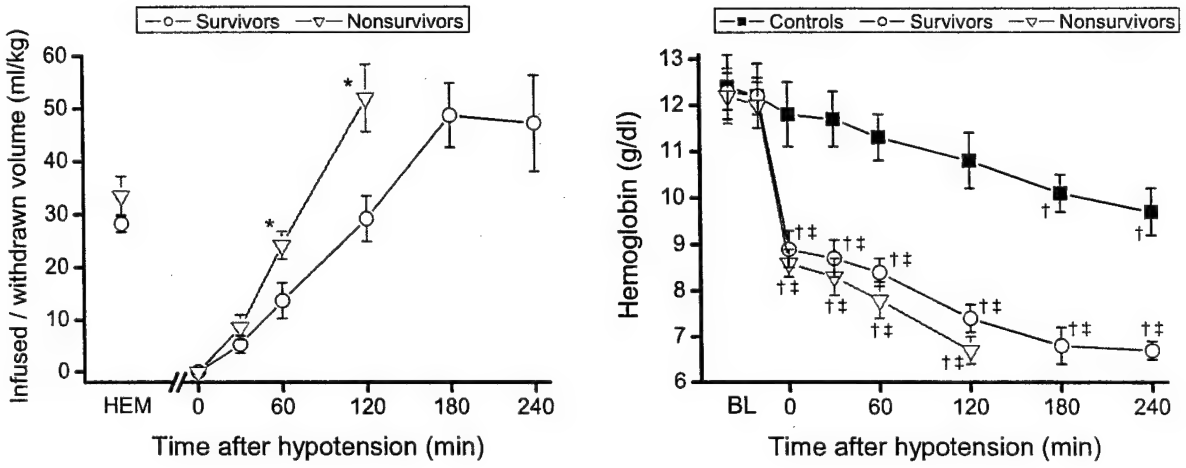

Survivors
Nonsurvivors
Infused / withdrawn volume (ml/kg)
60
50
40
30
20
10
0
HEM
0
60
120
180
240
Time after hypotension (min)
Controls
Survivors
Nonsurvivors
Hemoglobin (g/dl)
13
12
11
10
9
8
7
6
BL
0
60
120
180
240
Time after hypotension (min)

Figure 2

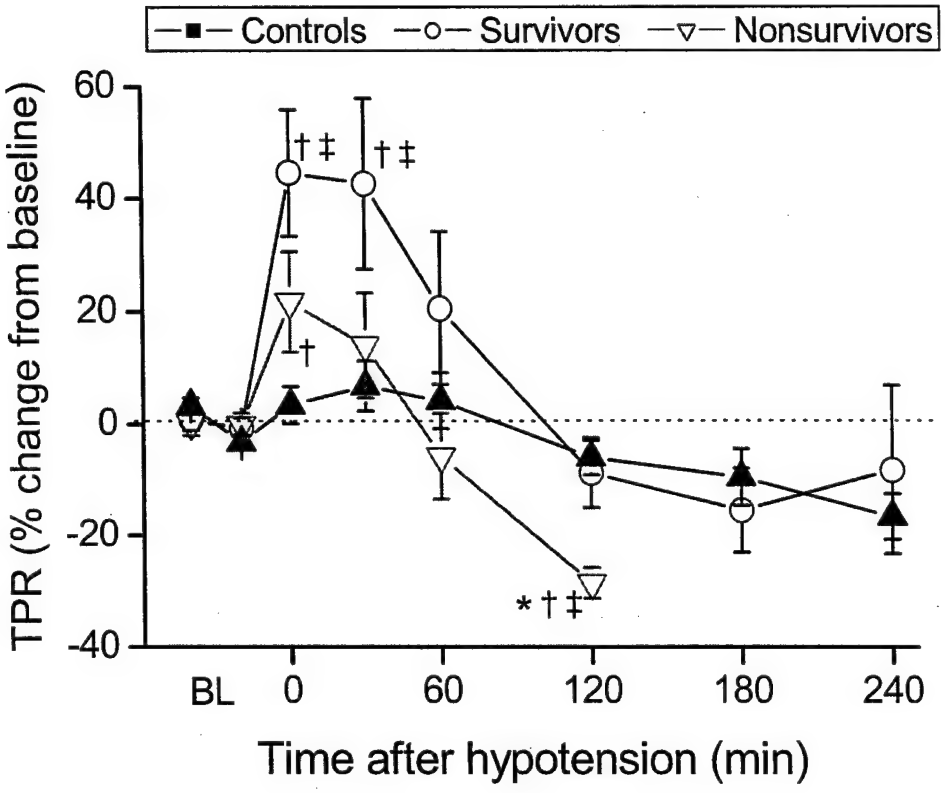

Controls
Survivors
Nonsurvivors
TPR (% change from baseline)
60
40
20
0
-20
-40
BL
0
60
120
180
240
Time after hypotension (min)

Figure 3

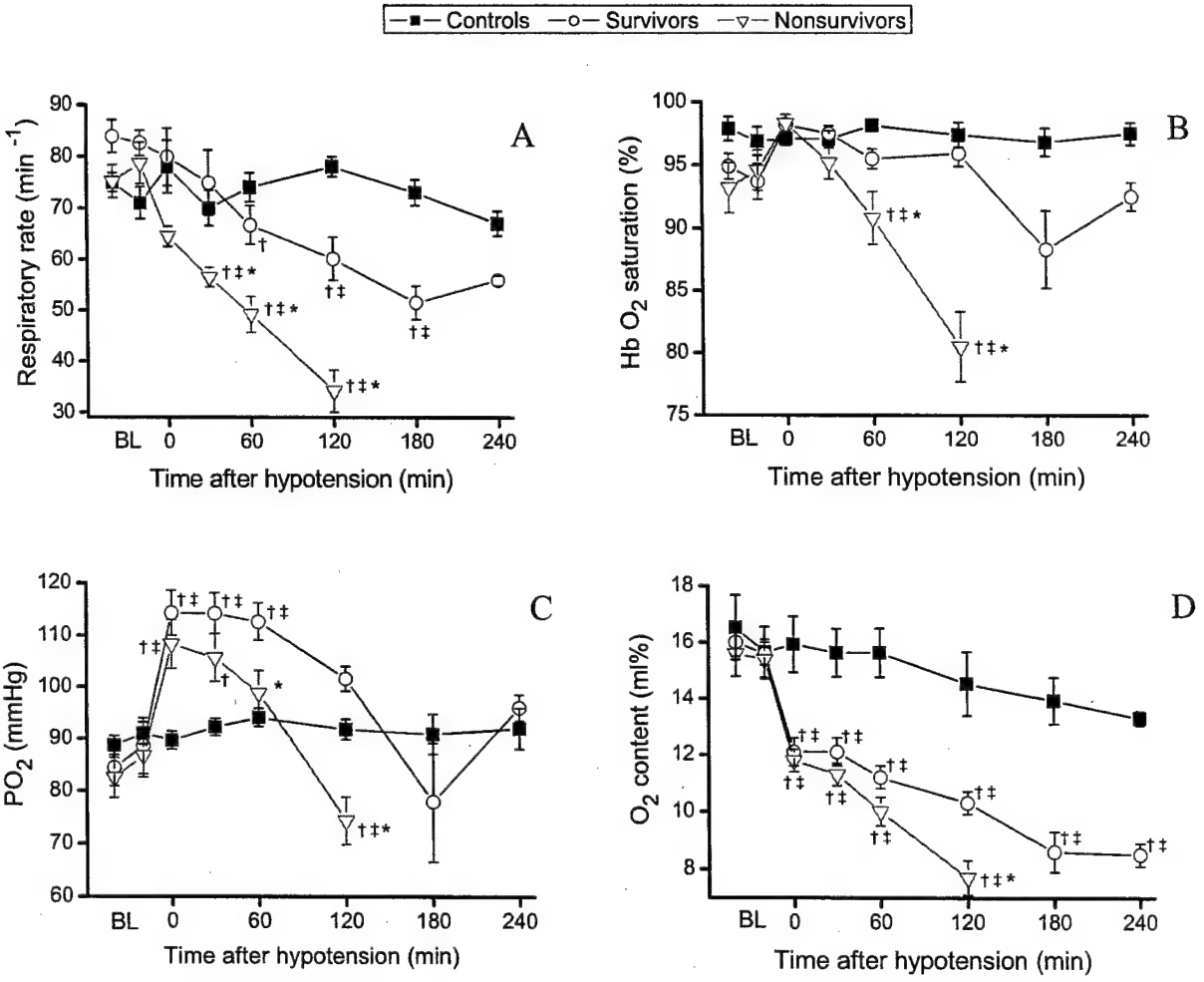

Controls
Survivors
Nonsurvivors
A
Respiratory rate (min -1)
Time after hypotension (min)
B
Hb O2 saturation (%)
Time after hypotension (min)
C
PO2 (mmHg)
Time after hypotension (min)
D
O2 content (ml%)
Time after hypotension (min)
BL
0
60
120
180
240

Figure 4

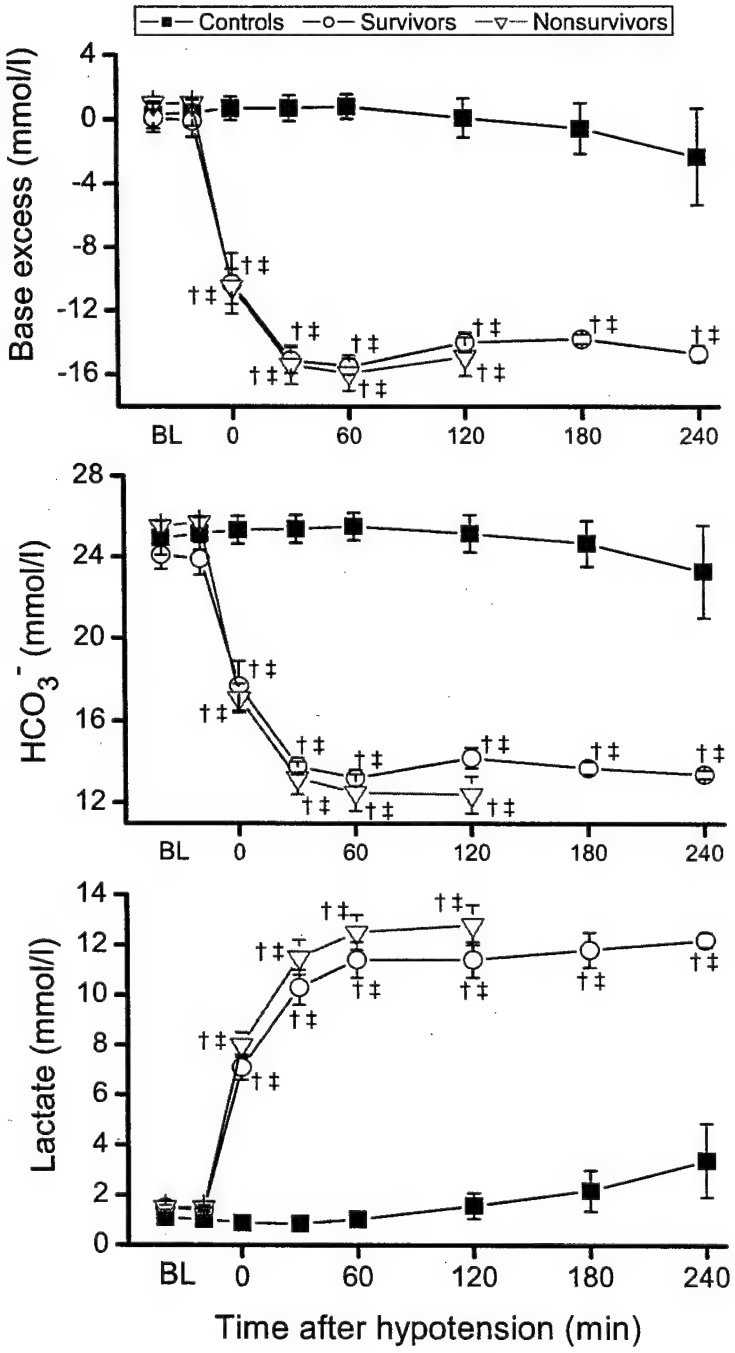

Controls
Survivors
Nonsurvivors
Base excess (mmol/l)
4
0
-4
-8
-12
-16
BL
0
60
120
180
240
HCO_3^- (mmol/l)
28
24
20
16
12
Lactate (mmol/l)
14
12
10
8
6
4
2
0
Time after hypotension (min)

Figure 5

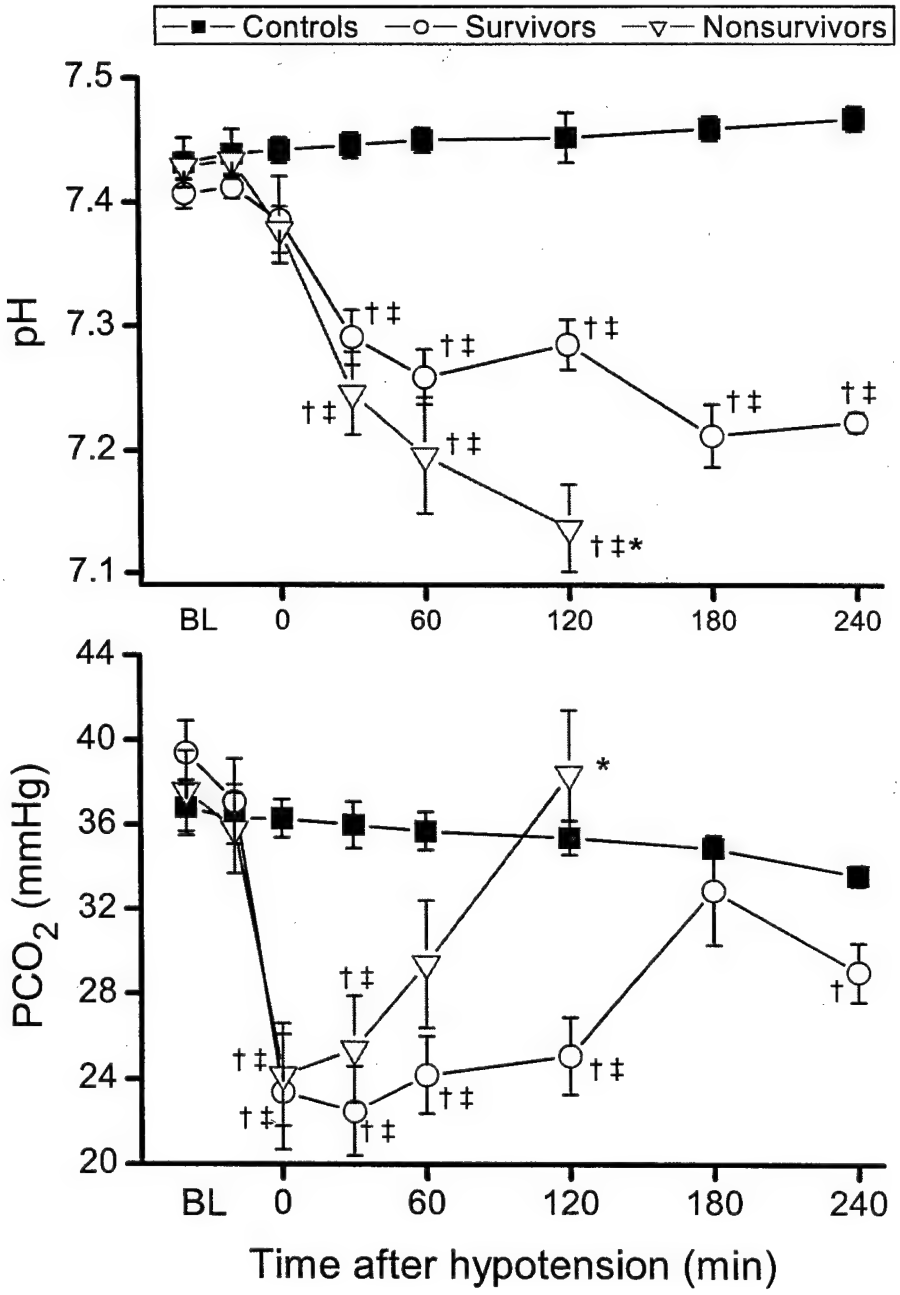

Controls
Survivors
Nonsurvivors
pH
7.5
7.4
7.3
7.2
7.1
BL
0
60
120
180
240
PCO2 (mmHg)
44
40
36
32
28
24
20
Time after hypotension (min)

Figure 6

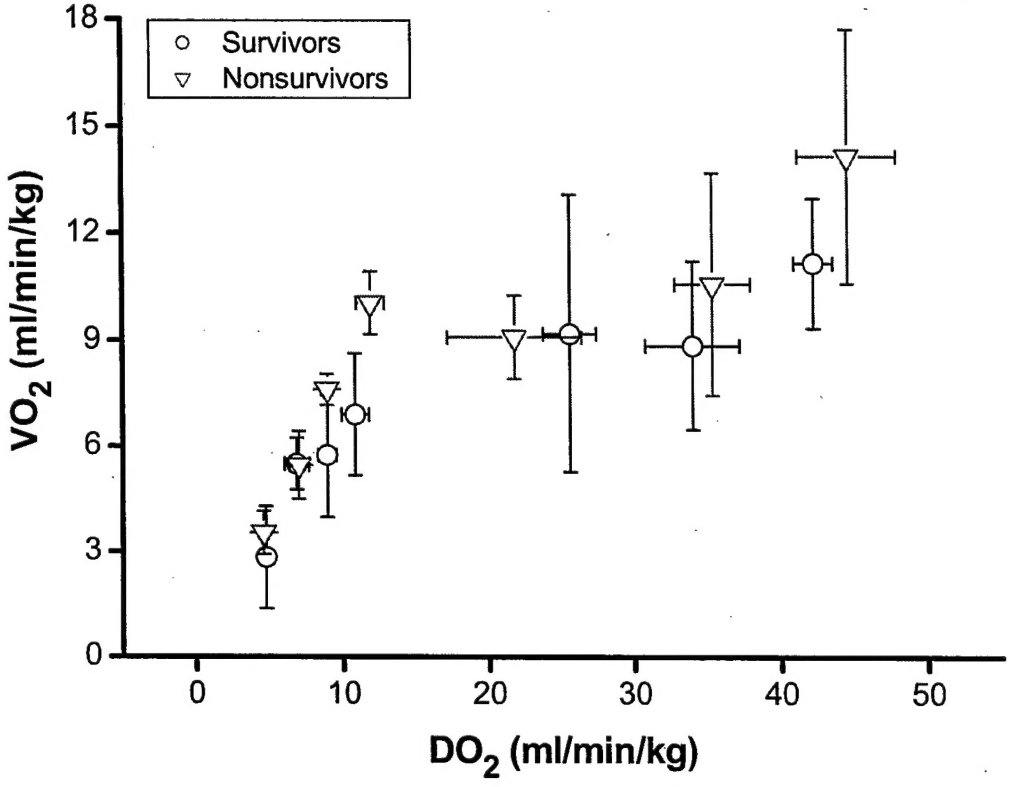

Survivors
Nonsurvivors
18
15
12
9
6
3
0
0
10
20
30
40
50
VO2 (ml/min/kg)
DO2 (ml/min/kg)

Figure 7

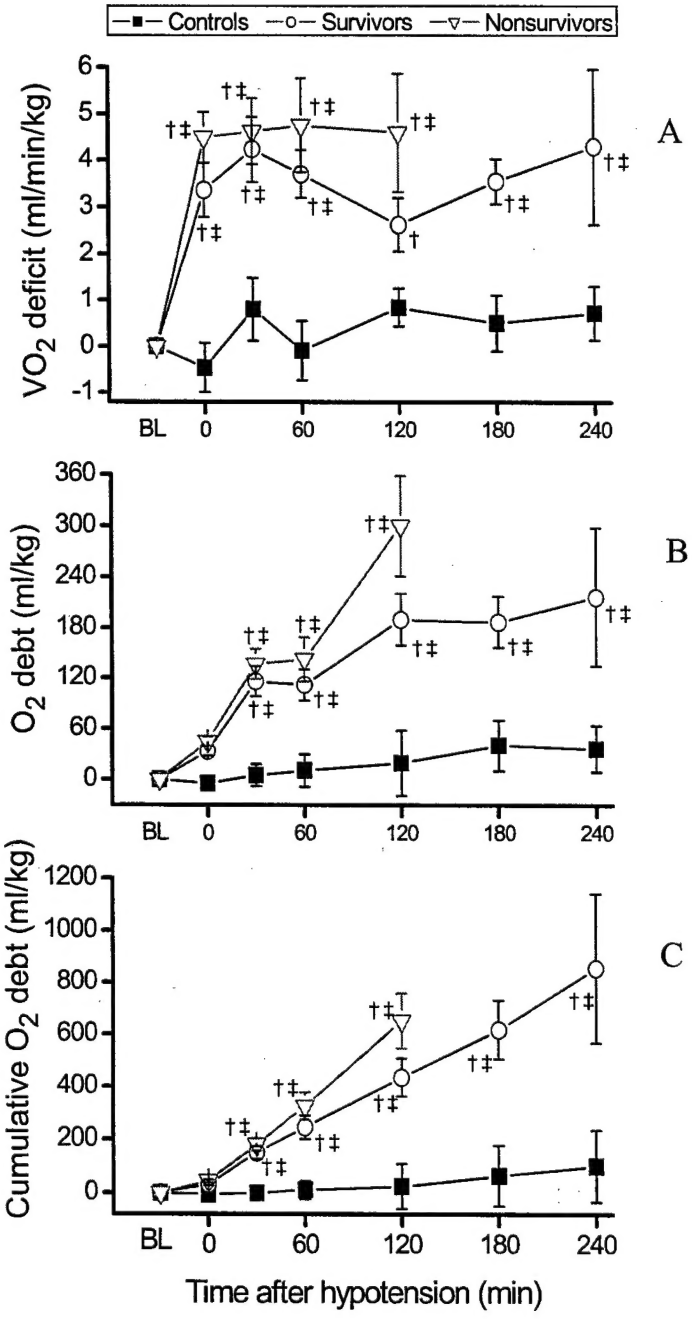

Controls
Survivors
Nonsurvivors
VO_2 deficit (ml/min/kg)
O_2 debt (ml/kg)
Cumulative O_2 debt (ml/kg)
A
B
C
BL
0
60
120
180
240
Time after hypotension (min)

Figure 8

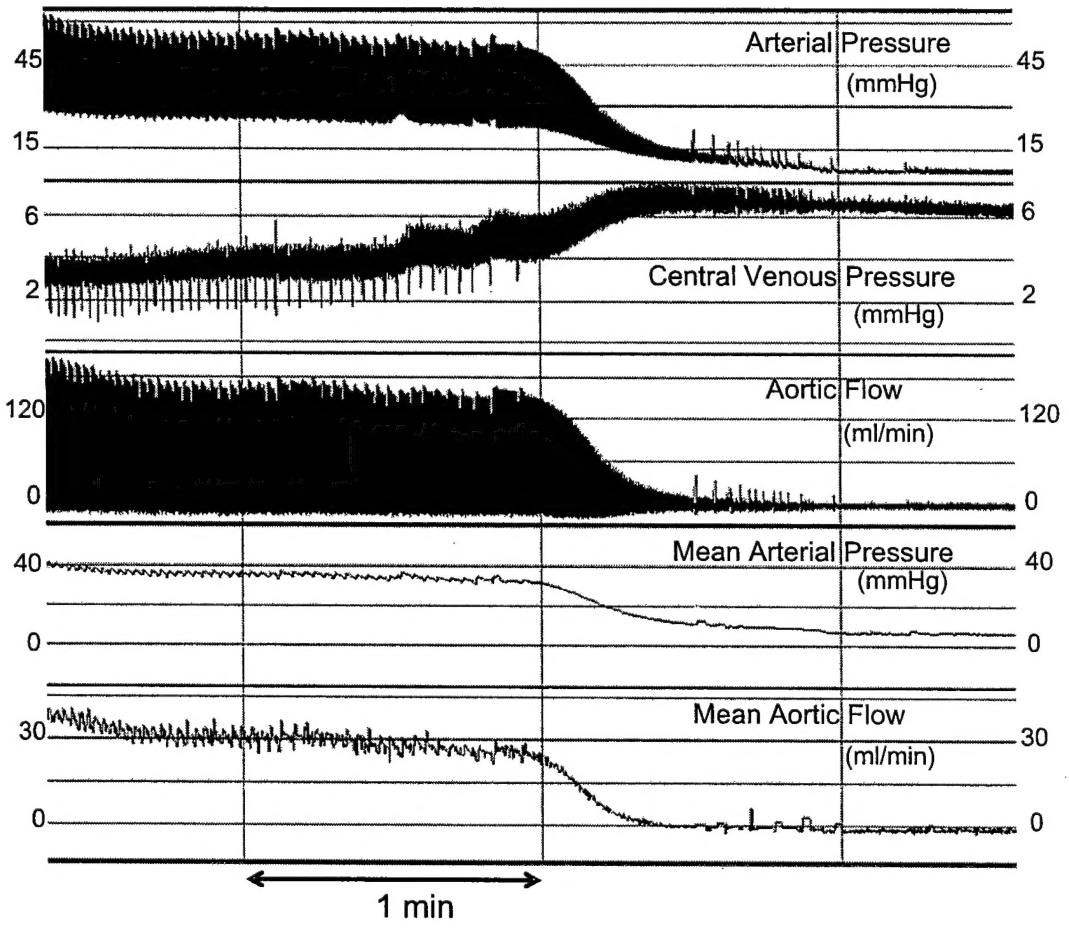
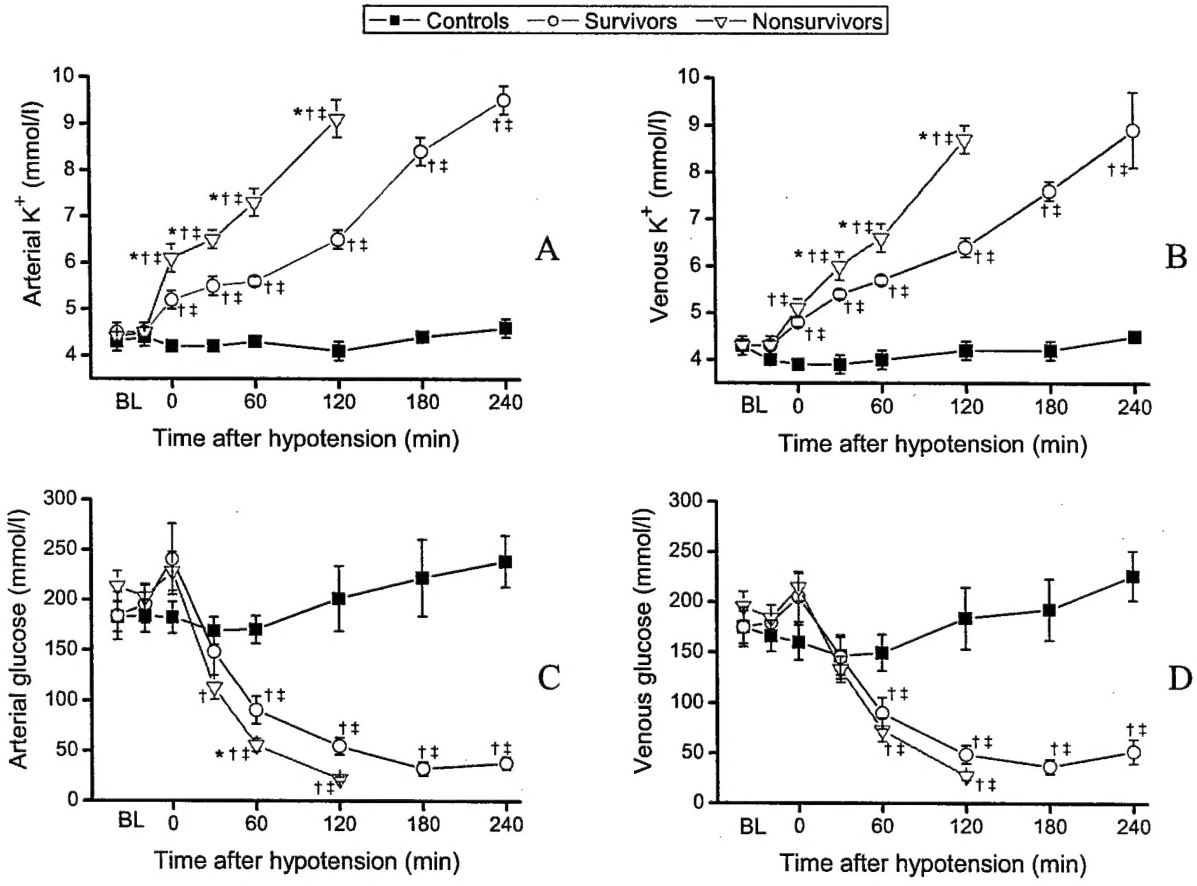

45
15
Arterial Pressure
(mmHg)
45
15
6
2
Central Venous Pressure
(mmHg)
6
2
120
0
Aortic Flow
(ml/min)
120
0
40
0
Mean Arterial Pressure
(mmHg)
40
0
30
0
Mean Aortic Flow
(ml/min)
30
0
1 min

Figure 9


Controls
Survivors
Nonsurvivors
Arterial K+ (mmol/l)
Venous K+ (mmol/l)
Arterial glucose (mmol/l)
Venous glucose (mmol/l)
Time after hypotension (min)
BL
0
60
120
180
240
A
B
C
D